Evolution Series UV-Vis Instruments

Evolution 200 Series Spectrophotometer

User Guide

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Thermo SCIENTIFIC

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Telephone: +1 608 273 5017
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For Research Use Only. This instrument or accessory is not a medical device and is not intended to be used for the prevention, diagnosis, treatment or cure of disease.

WARNING Avoid an explosion or fire hazard. This instrument or accessory is not designed for use in an explosive atmosphere.

Contents

Introduction

This document covers the Thermo Scientific™ INSIGHT™ software and the Evolution™ 200 series UV-Visible spectrophotometers and accessories.

Contents

About the Software and Hardware

Contents

Overview

The Evolution 200 series UV-visible spectrophotometers integrate advanced hardware features with the power and flexibility of a wide range of Smart Accessories™. The 200 series spectrophotometers include:

- Evolution 201
- Evolution 220
- Evolution 260 Bio

For a list of sampling and other accessories available for these instruments, see Evolution 200 Series Accessories.

All instruments include our INSIGHT software for data collection and analysis. The INSIGHT software provides five modes of operation:

- **Fixed**, to measure the light passing through the sample at one or more wavelengths.
- **Scan**, to measure the light that passes through the sample over a range of wavelengths.
- **Quant**, to set up and perform quantitative analyses of sample data.
- **Rate**, to make time- and temperature-based kinetics measurements.
- **Live Display**, for quick measurements and simplified data collections in Fixed or Scan mode.

The Evolution 260 Bio systems also have operating modes for the common biological applications, including Nucleic Acid and Protein A280. See INSIGHT Bio Applications for more information.

Setting Up the Instrument

The spectrophotometer can be run from the optional touchscreen (Local Control) or from a Windows®-compatible computer connected to the instrument (Computer Control). Local Control instruments include an embedded computer preloaded with software. Computer Control instruments must be connected to an external computer with the proper software installed. All instruments include a keypad.

For instructions to set up a Computer Control or Local Control spectrophotometer and perform basic operations using the keypad, see Using the Spectrophotometer.

Before Using the Spectrophotometer

The spectrophotometer contains precise optical components. Handle it carefully. Before using the system, review the Site Preparation and Safety information and Operating Precautions.

About This Document

Contents

Organization

This document has the following main sections:

Section	Description
Introduction	Overview of the instrument and this document plus information about registering your instrument, contacting us, and the system warranty.
Evolution 200 Series Spectrophotometers	Complete instructions for using and maintaining the instrument.
INSIGHT Software	Complete instructions for using software features other than biological applications and Performance Verification (see below).
Bio Applications	Instructions for using biological applications to analyze samples.
Accessories	Complete instructions for using accessories with the instrument.
Performance Verification	Instructions for setting up and running tests to check the performance of the instrument.
INSIGHT Security	Optional software that adds features for digitally signing files and verifying digital signatures.

Conventions

This document uses the following conventions:

WARNING Indicates a hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION Indicates a hazardous situation which, if not avoided, could result in minor or moderate injury.

NOTICE Follow instructions with this label to avoid damaging the system hardware or losing data.

Note Contains helpful supplementary information.

Tip Provides helpful information that can make a task easier.

Contacting Us

For U.S. Technical Support, please contact:

Thermo Fisher Scientific
5225 Verona Road
Madison, WI 53711-4495 U.S.A.
Telephone: 1 800 532 4752
E-mail: us.techsupport.analyze@thermofisher.com

For International Support, please contact:

Thermo Fisher Scientific
Telephone: +1 608 273 5017
E-mail: support.madison@thermofisher.com

Note Please have the instrument serial number available when you contact us.

E-mailing Data Files

If you have questions about your data, you can send it to us as an archived workbook file (*.iwbk). Please send us the appropriate file as an e-mail attachment. See the description of E-mail Current Workbook in Menus and the description of E-mail in Data Store Tab for more information.

Note Please do not send exported report files or user-defined workbooks—these do not contain the information we need.

Product Warranty

Warranties herein are for products manufactured by Thermo Fisher Scientific or its authorized dealers.

Thermo Fisher Scientific warrants that this product is free from defects in labor and materials and shall conform to its product specifications as defined in the product user documentation.

This warranty covers parts (except those specified below) and labor, and applies only to equipment which has been installed and operated in accordance with the documentation supplied by Thermo Fisher Scientific, and which has been serviced only by authorized Thermo Fisher Scientific dealers or service personnel. This warranty does not apply to equipment and accessories that have been modified or tampered with in any way, misused, or damaged by accident, neglect, or conditions beyond Thermo Fisher Scientific's control.

Related Topics

Warranty Period

Items Not Covered by Warranty

Warranty Repair

Shipping Cost for Items Covered by Warranty

Update Policy

Warranty Period

With the exception of SPECTRONIC™ 20+, SPECTRONIC 20D+, GENESYS™ 20 and the Helios™ Epsilon spectrophotometers, all UV Visible spectrophotometers come with a standard warranty that is fourteen months from the date of shipment or twelve months from the date of installation (whichever occurs first). The replacement parts and spare components are warranted for 90 days from the date of shipment. If such parts are intended for long term storage, it is recommended that their functionality is tested immediately upon receipt and any problems reported to Thermo Fisher Scientific within the above specified warranty period.

The following warranties apply to instrument light sources:

1. Xenon lamps are warranted against failure for a period of three (3) years.
2. Tungsten and deuterium lamps will be replaced under warranty if they fail on the initial power-up or within the first month of operation.

Thermo Fisher Scientific reserves the right to request the return of failed components that are being replaced under warranty.

User should complete and return the Product Registration card, and retain proof of delivery date.

Items Not Covered by Warranty

This warranty does not apply to glassware, expendable components, peripheral devices or accessories not manufactured by Thermo Fisher Scientific. The manufacturer of these products may offer specific warranties for such parts and components.

Cleaning and calibration service is not covered by this warranty.

Warranty Repair

In the event of failure within the warranty period, Thermo Fisher Scientific will at Thermo Fisher Scientific's option, repair or replace the product not conforming to this warranty. There may be additional charges, including freight, for warranty service performed in some countries. For service, call Thermo Fisher Scientific (or its authorized dealer outside the United States and Canada). Thermo Fisher Scientific reserves the right to ask for proof of delivery date.

Warranty repairs for UV Visible instruments may vary per product line and region. UV Visible products manufactured by Thermo Fisher Scientific are covered by a "return-to-factory" parts and labor warranty. In some regions, on-site warranties, extended warranties and service contracts may be purchased for all products.

In the event that on-site warranty service is required, and no on-site service contract is active on this product, the customer is responsible for any travel expenses, labor costs, and additional expenses which may be incurred in the performance of on-site warranty service.

Please contact your dealer or Thermo Fisher Scientific Technical Service department for further information. A Return Authorization Number must be obtained from Thermo Fisher Scientific Technical Service before returning any product for in-warranty repair or replacement.

Shipping Cost for Items Covered by Warranty

It is customer's responsibility to pay for the shipment of the product under warranty to Thermo Fisher Scientific.

This warranty covers return shipment (standard ground) charges for returning the repaired or replaced product to the customer.

Update Policy

Thermo Fisher Scientific may, from time to time, revise the performance of its products, and in doing so incur no obligation to furnish any such revisions to any Thermo Fisher Scientific customer.

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Site Preparation and Safety

Before using the system, read the site preparation and safety manual on the provided documentation CD. Always follow the safety precautions in that manual and in this document when using the system.

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Evolution 200 Series Spectrophotometers

The Evolution™ 200 series UV-Visible spectrophotometers include three models:

- Evolution 201
- Evolution 220
- Evolution 260 Bio (includes biological applications).

Contents

Safety Considerations

Each person using the spectrophotometer should read the general safety information in the Site and Safety Information guide (see the documentation CD) and the instrument-specific safety information provided here.

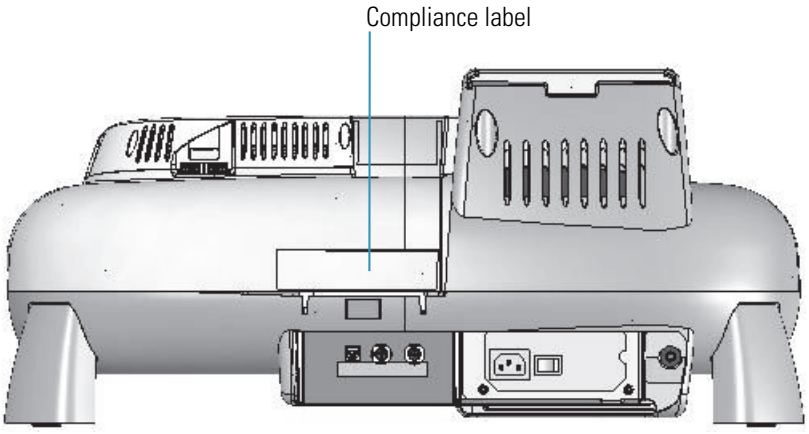
Contents

WARNING Do not operate this system without following the safety precautions described in this manual and the documentation that came with your system.

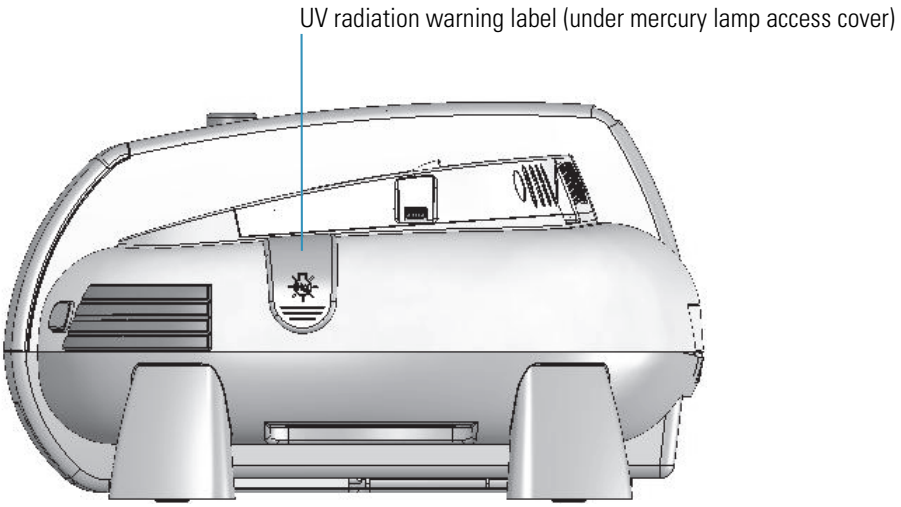
Safety Label Locations

This section shows the locations of safety labels on the spectrophotometer. If there are questions or problems with the safety labels, contact us using the information provided at the beginning of this document.

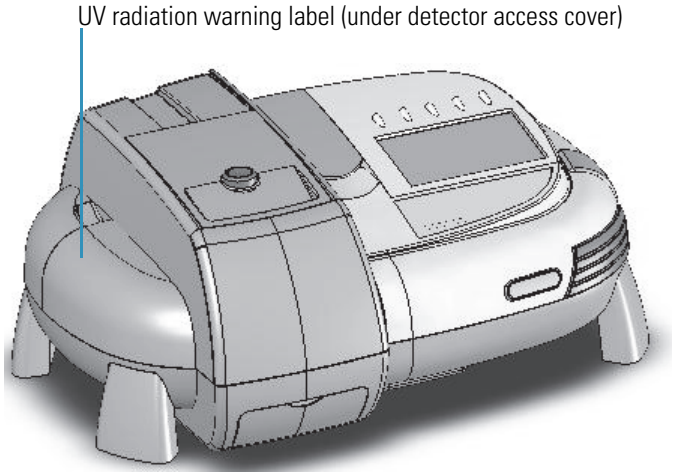
Back view



Right side view



Left side view

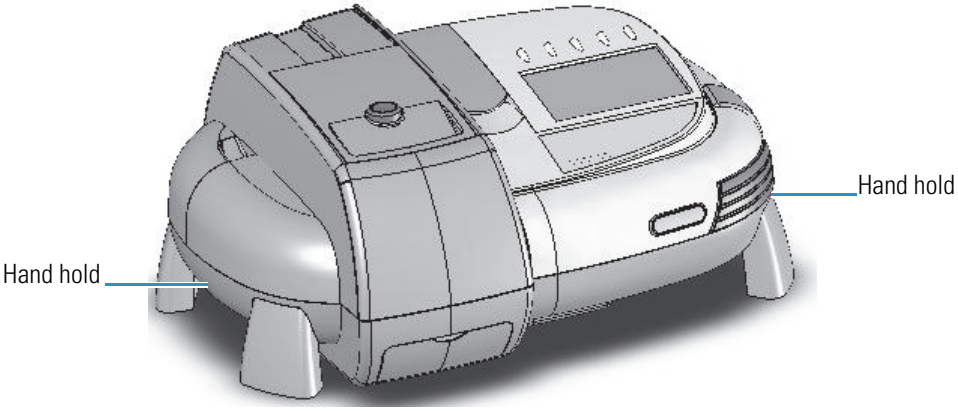


Related Topics

Removing and Installing the Detector Module

Lifting or Moving the Instrument

To avoid risk of injury, use proper techniques when lifting or moving the instrument or other system components.



Operating Precautions

The spectrophotometer contains precise optical components. Handle it carefully and follow these precautions:

- Do not allow moisture to leak into the instrument interior.
- Wipe off spilled chemicals immediately.
- Do not drop the instrument.
- Protect the instrument from mechanical shock.
- Protect the instrument from dust.

Spectrophotometer Basics

Contents

Spectrophotometer Components

The following illustration identifies major components visible on the outside of a typical spectrophotometer. (Some components may not be present on all instruments.)

The z-height (distance from the bottom of the cell to the center of the light beam) for the spectrophotometer is 8.5 mm.

Related Topics

Opening and Closing the Sample and Reference Compartment Doors

Local Control or Computer Control

INSIGHT Software

Keypad

Touchscreen

Opening and Closing the Sample and Reference Compartment Doors

To open the sample compartment, press the button on its door. To close the compartment, slide the door forward until it latches.

To access the reference compartment, lift or lower the door.

Related Topics

Sample Compartment Front Panel

Tubing Access Panel

Local Control or Computer Control

The spectrophotometer can be run from the optional touchscreen (Local Control) or from a Windows®-compatible computer connected to the instrument (Computer Control). Local Control instruments include an embedded computer preloaded with software. Computer Control instruments must be connected to an external computer with the proper software installed.

Related Topics

Local Control Instruments

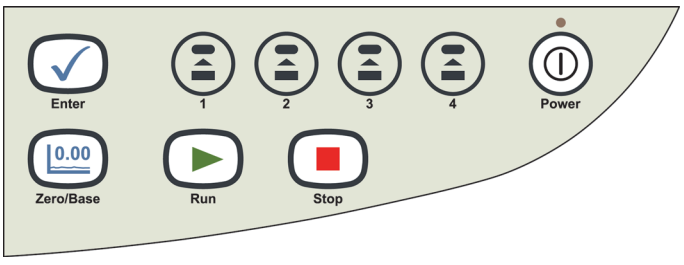
Computer Control Instruments

INSIGHT Software

All instruments include our INSIGHT™ software for data collection and analysis. See INSIGHT Software.

Keypad

All instruments include the keypad shown below. For a list of keypad functions, see Using the Spectrophotometer.



Related Topics

Keypad Operation

Touchscreen

The touchscreen is included only on Local Control versions of the spectrophotometer.

Related Topics

Touchscreen keyboard

Cleaning the Touchscreen

Connectors

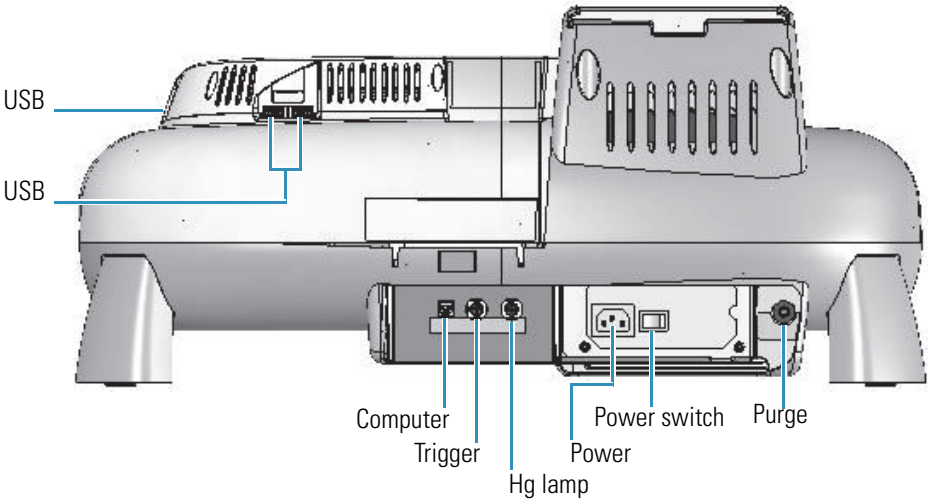
This section shows the locations of the connectors inside and outside the spectrophotometer.

Contents

Outside Cover

This illustration shows the connectors on the instrument outside cover.

Back view



Label	Function
Computer	Dedicated USB port for connecting an external computer. See Computer Control Instruments.
Hg lamp	Connects to the optional Mercury Lamp accessory.
Power	Connects to main power cord.
Power switch	System power On/Off switch.
Purge	Connects to optional purge source. See Purging the Sample Compartment.

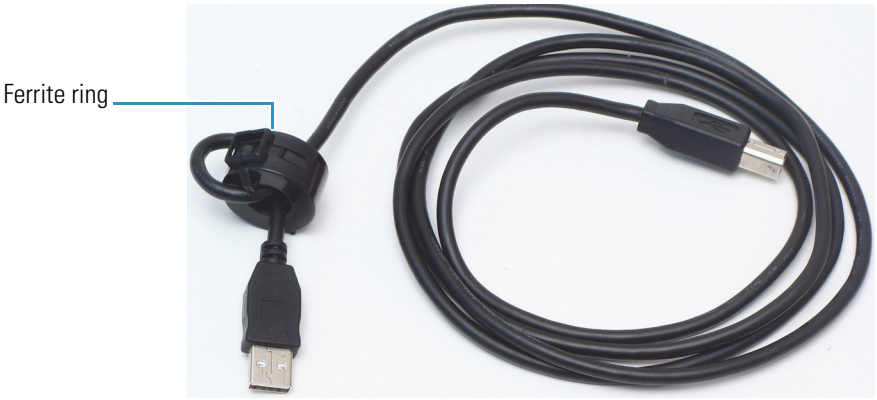
Label	Function
Trigger	Connects to optional accessories that accommodate external trigger input or output. For more information, find the *Integrated Fiber Optic Module User Guide* in the documentation media. INSIGHT can be used to send a 3.3V TTL output signal that triggers an installed accessory to begin an operation. The signal can be sent at the start or end of a measurement. See **Options > Preferences > Triggering**. INSIGHT accepts a contact closure input trigger (equivalent to pressing Run on the instrument keypad).
USB	General purpose USB connectors (Local Control systems only). See below for proper shielding of USB cables.

WARNING Avoid shock hazard. Power off the spectrophotometer and disconnect the power cord from the wall outlet or power strip before disconnecting the power cord from the spectrophotometer.

The system supports these USB devices:

- External USB mouse
- External USB keyboard
- Portable USB memory
- Windows-compatible USB printer

To ensure proper shielding of USB devices, install a Ferrite ring (samples provided) on the end of any USB cable connected to the instrument. Loop the cable once and clamp the ring over the loop as shown below.



Note The spectrophotometer does not support high power USB devices.

Related Topics

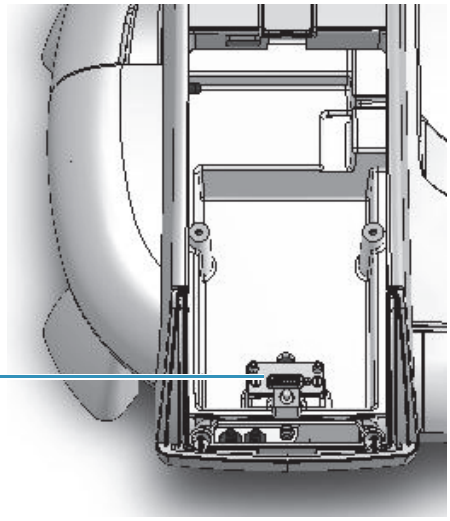
Triggering

Purging the Sample Compartment

Computer Control Instruments

Inside the Sample Compartment

This illustration identifies the connectors inside the sample compartment.



Related Topics

Installing a Smart Accessory

Removable Panels

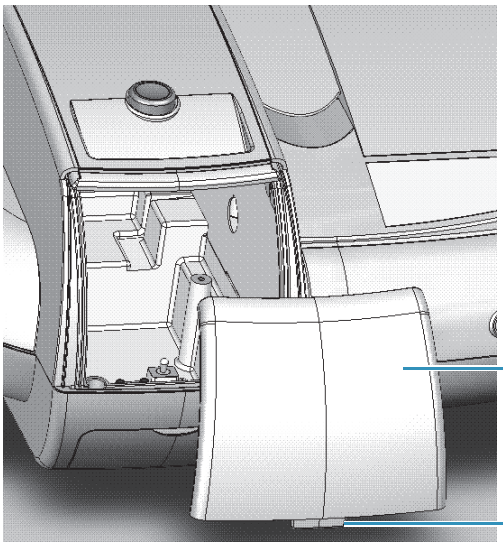
This section covers panels that can be removed to allow access to features on the instrument.

Contents

Sample Compartment Front Panel

To remove the front panel, open the sample compartment door and then pull the panel forward and up to free it.

To replace the panel, align the tab on the bottom edge with the slot in the instrument cover and press the panel in place.



Related Topics

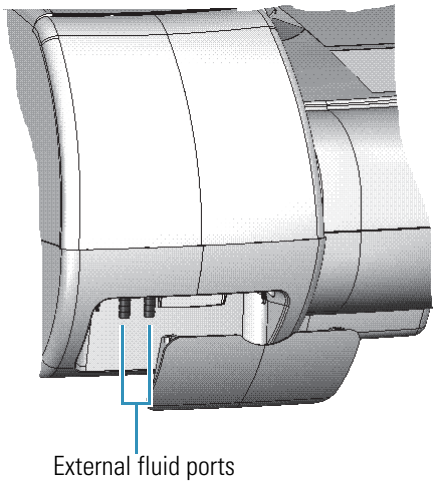
Installing a Manual Accessory

Installing a Liquid Thermostatted Accessory

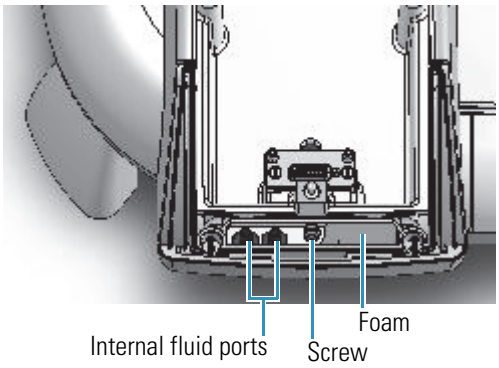
Installing a Smart Accessory

Tubing Access Panel

To access external ports for routing fluid from a recirculator to the instrument, remove the panel below the sample compartment.



Internal fluid ports for routing fluid from the instrument to an installed accessory are located inside the sample compartment.



The connectors fit 1/4-inch internal diameter tubing. An adapter may be required to connect the tubing to the accessory or recirculator. Purchase adapters separately.

Cords and cables for installed accessories can also be routed behind this panel. Push the cord through the foam and the opening below it. The panel itself can be removed temporarily to accommodate a large connector. To remove the panel, loosen the screw.

Related Topics

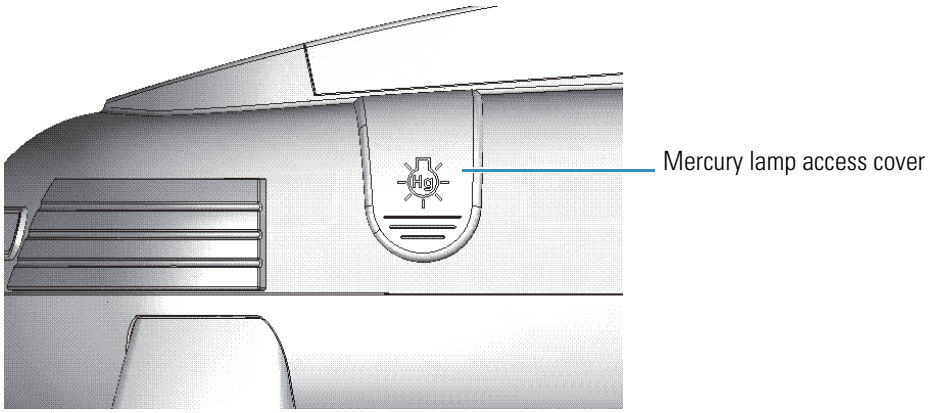
Installing a Liquid Thermostatted Accessory

Mercury Lamp Access Cover

WARNING Avoid UV radiation hazard in the mercury lamp compartment. Protect eyes and skin from exposure.

Lift the cover to install the optional Mercury Lamp. For more information, refer to the *Mercury Lamp User Guide* in the documentation media.

Right side view

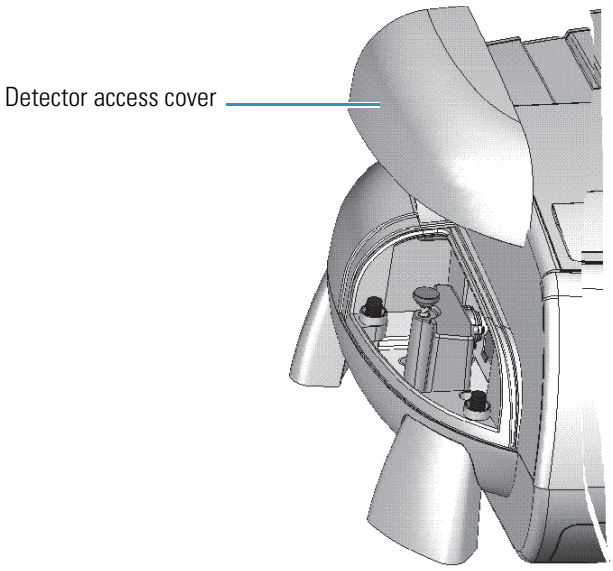


Detector Access Cover

Some accessories require removing the instrument detector module. The module is beneath the detector access cover shown below. Avoid UV radiation hazard in the detector compartment. Protect eyes and skin from exposure.

CAUTION Safety glasses with side shields or goggles with solid side pieces are the only equipment that provides adequate eye protection against direct and reflected UV light.

Left side view



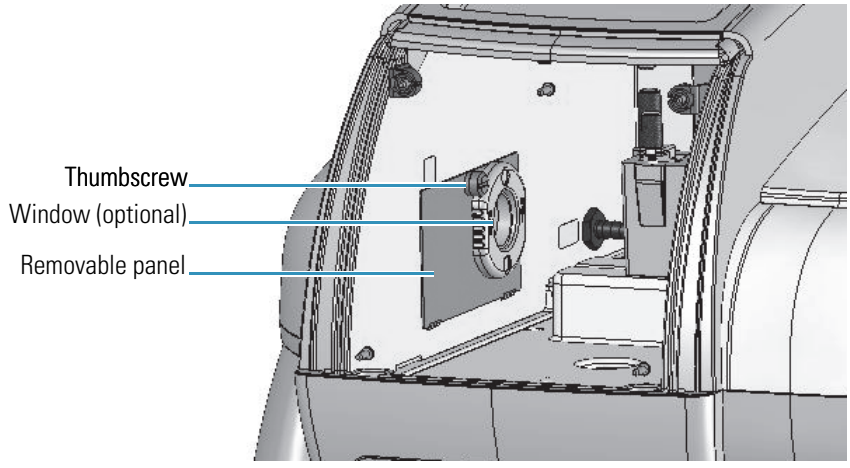
Related Topics

Removing and Installing the Detector Module

Detector Access Panel

Detector Access Panel

To remove the panel between the detector and sample compartment, remove the sample compartment window and then loosen the thumbscrew.



To replace the panel, hook the tabs on the bottom edge over the slots in the sample compartment side wall and tighten the thumbscrew.

Related Topics

Removing and Installing the Detector Module

Detector Access Cover

Installing Sample Compartment Windows

Corrosion Protection

The spectrophotometer contains precise optical components that may be damaged by a corrosive environment. Before using the instrument to analyze volatile, corrosive or caustic samples, install the optional sample compartment windows and, if necessary, purge the sample compartment.

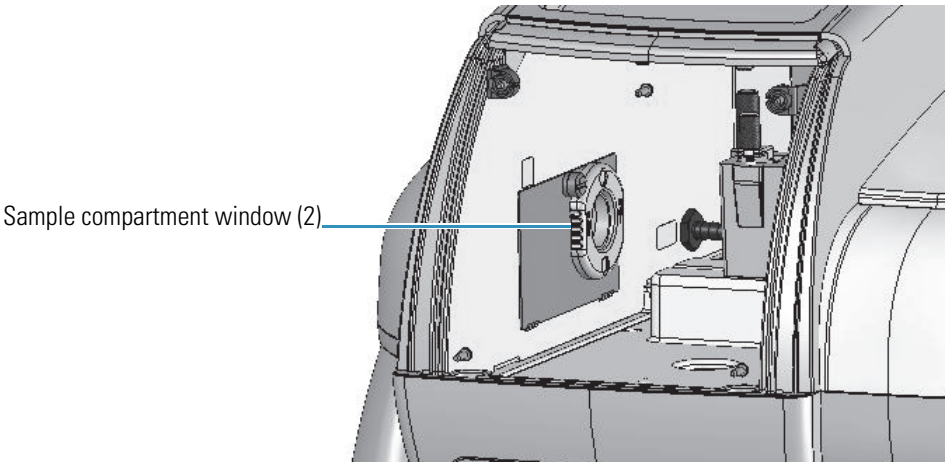
Related Topics

Installing Sample Compartment Windows

Purging the Sample Compartment

Installing Sample Compartment Windows

The optional windows attach to the sample compartment side walls using magnets.



NOTICE The warranty does not cover damage to internal optics or electronics caused by failure to use sample compartment windows.

Related Topics

Purging the Sample Compartment

Purging the Sample Compartment

Purging the sample compartment removes moisture from the sampling area for subambient temperature-controlled measurements. When purging the instrument, install sample compartment windows in addition to purge.

Related Topics

Installing Sample Compartment Windows

Selecting a Purge Gas

Installing Purge

Selecting a Purge Gas

Use dry air or dry nitrogen to reduce or eliminate condensation. The purge gas must be free of moisture, oil and other reactive materials. To remove particulate matter and oil, install a 10-micrometer filter.

WARNING *Never* use a flammable gas to purge the instrument.

Related Topics

Installing Sample Compartment Windows

Installing Purge

Installing Purge

Use 1/4-inch internal diameter tubing to connect a purge gas source to the purge fitting on the back of the spectrophotometer.

Related Topics

Installing Sample Compartment Windows

Selecting a Purge Gas

Installing and Removing Accessories

Contents

Standard Cell Holders

The instrument includes one sample and one reference cell holder. For a list of optional cell holders and other accessories, click the **Evolution 200 Series Accessories** button in the documentation media.

Related Topics

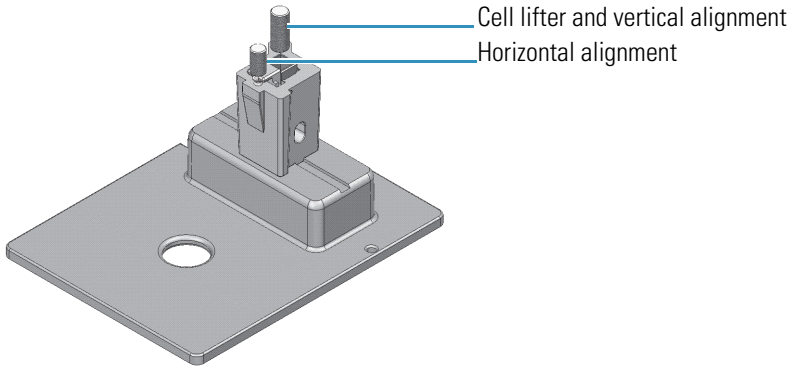
10 mm Rectangular Sample Cell Holder

Rectangular Reference Cell Holder

10 mm Rectangular Sample Cell Holder

This single-cell holder is mounted on the standard baseplate and accommodates standard 10 mm pathlength cells. See Installing a Manual Accessory.

Use the cell lifter to raise the cell for easy removal. This cell holder allows fine adjustment of the vertical and horizontal position of the cell. This feature is useful for aligning reduced volume and microcuvettes with the light beam. See z-height.



Related Topics

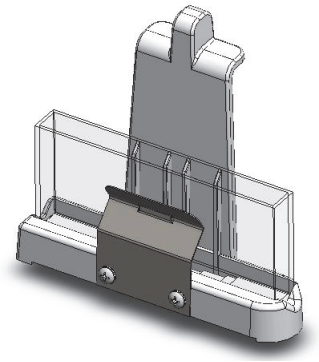
Rectangular Reference Cell Holder

Spectrophotometer Z-height

Rectangular Reference Cell Holder

For measurements with a reference sample, install a reference cell holder in the reference position. See Installing a Reference Cell Holder.

The standard reference holder accommodates rectangular reference cells with these pathlengths: 10 mm, 20 mm, 40 mm, 50 mm and 100 mm.



Related Topics

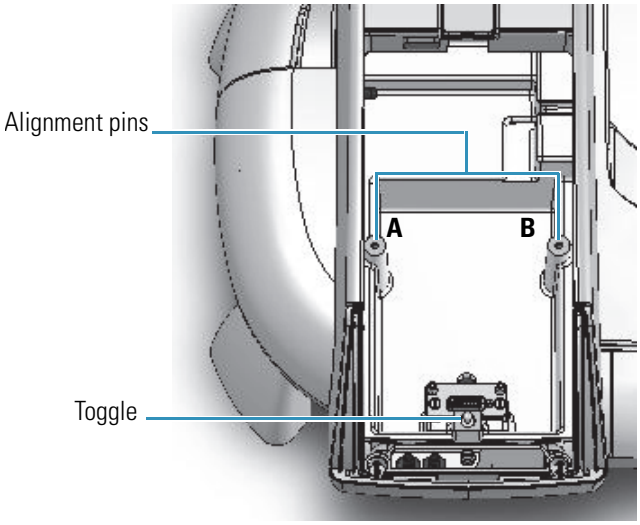
10 mm Rectangular Sample Cell Holder

Installing a Manual Accessory

Manual sampling accessories are mounted on a common baseplate. The kinematic mount ensures correct alignment of the accessory within the instrument.

It is not necessary to power off the instrument while installing or removing a manual accessory.

Sample compartment top view



❖ To install a manual accessory

1. Open the sample compartment door. See Opening and Closing the Sample and Reference Compartment Doors.
2. Remove any accessory from the compartment (see the instructions below).
3. Position the accessory so the two alignment holes on its baseplate fit over pins A and B in the sample compartment.
4. Gently push down on the front edge of the baseplate until it snaps in place under the toggle.
5. Close the sample compartment door. See Opening and Closing the Sample and Reference Compartment Doors.

For operating instructions and installation details, see the user guide for the accessory.

❖ To remove a manual accessory

1. Use the handle or finger hole on the accessory baseplate to release the plate from the toggle.
2. Lift the plate off the alignment pins and remove the accessory from the sample compartment.

Related Topics

Installing a Liquid Thermostatted Accessory

Installing a Smart Accessory

Installing a Reference Cell Holder

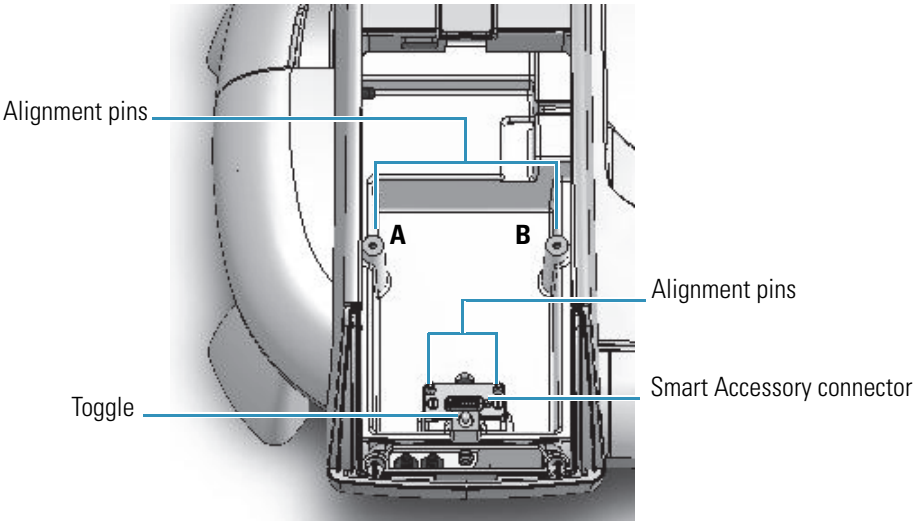
Installing a Smart Accessory

Smart Accessories include cell changers and sample holders that feature auto recognition, smart alignment and serial number reporting. Smart Accessories ensure software methods are properly configured.

These accessories install in the sample compartment. A connector under the accessory baseplate provides data communication and power.

It is not necessary to power off the instrument while installing or removing a Smart Accessory.

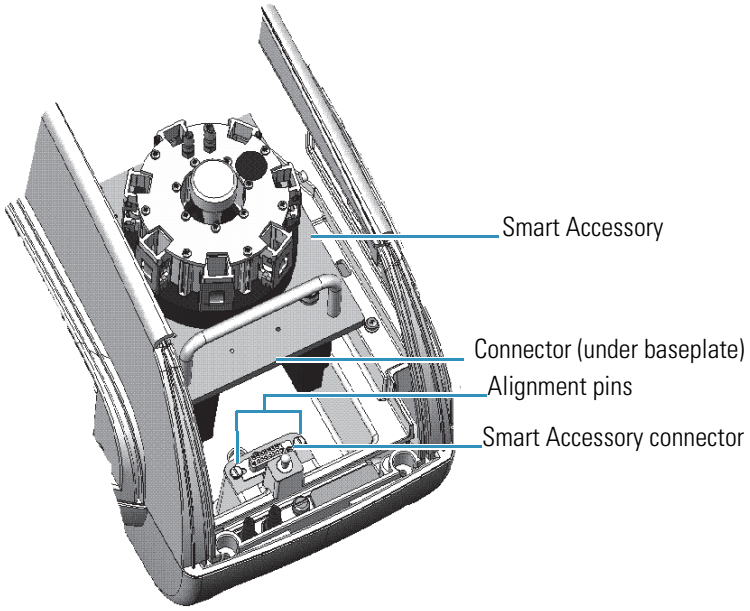
Sample compartment top view



❖ To install a Smart Accessory

1. Open the sample compartment door. See Opening and Closing the Sample and Reference Compartment Doors.
2. Remove the sample compartment front panel. See Sample Compartment Front Panel.
3. Remove any cell holder or accessory. See To remove a Smart Accessory.
4. Insert the accessory.
   a. Grasp the accessory by the handles and lower it into the sample compartment aligning the connector under the baseplate with the connector in the sample compartment floor.

Use the alignment pins on either side of the connector as guides.



b. Line up the two holes at the back of the accessory with pins A and B in the sample compartment.

c. Press down on the front of the accessory to secure the connection.

The software displays a prompt to initialize the accessory.

CAUTION Avoid pinch hazard. Keep hands and objects clear of the accessory during initialization.

5. Replace the sample compartment front panel. See Sample Compartment Front Panel.
6. Close the sample compartment door. See Opening and Closing the Sample and Reference Compartment Doors.
7. Click **OK** to initialize the accessory.

   Initialization reads information about the accessory and, for cell changers, moves it to position 1.

For operating instructions and installation details, see the user guide for the accessory.

❖ To remove a Smart Accessory

1. Open the sample compartment door. See Opening and Closing the Sample and Reference Compartment Doors.
2. Remove the sample compartment front panel. See Sample Compartment Front Panel.
3. Grasp the handles and pull the accessory up and out of the sample compartment.

The software confirms the accessory has been removed.

4. Replace the sample compartment front panel. See Sample Compartment Front Panel.
5. Close the sample compartment door. See Opening and Closing the Sample and Reference Compartment Doors.

Related Topics

Installing a Liquid Thermostatted Accessory

Installing a Manual Accessory

Installing a Reference Cell Holder

Installing a Liquid Thermostatted Accessory

A liquid thermostatted accessory has ports and internal chambers for circulating fluids through the unit for temperature-controlled measurements. Other accessories circulate fluids to cool heated components.

The Recirculator Tubing Kit provides parts for connecting an accessory to a temperature controller or fluid recirculator.

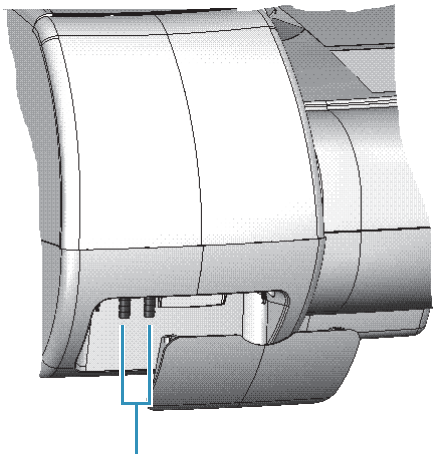
❖ To install a thermostatted accessory

1. Install the accessory.

   See Installing a Manual Accessory or Installing a Smart Accessory.

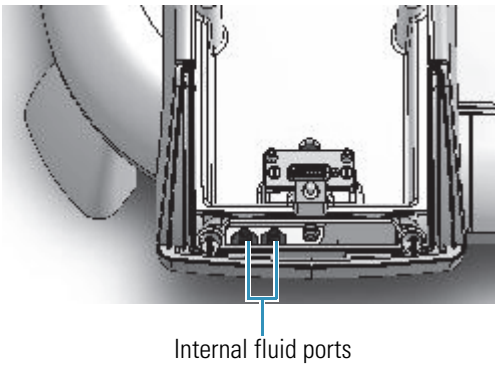
2. Remove the tubing access panel. See Tubing Access Panel.
3. Use 1/4-inch internal diameter tubing to connect the temperature controller, water supply or recirculator to the external fluid ports on the instrument.

If the connectors on the temperature controller, water supply or recirculator are a different size, use a tubing adapter.



4. Use additional tubing and adapters (not included), if necessary, to connect the fluid ports inside the sample compartment to the ports on the accessory.

   For a cell changer, set the changer to position 1 before connecting the tubing to the ports on the accessory. Use just enough tubing to allow the cell changer to move through all possible positions. This requires approximately 10 inches (25 cm) of tubing for each port.



Note Ensure the tubing does not obstruct the light beam or prevent the sample compartment door from closing. If necessary, secure the tubing using the supplied clips.

Related Topics

Installing a Smart Accessory

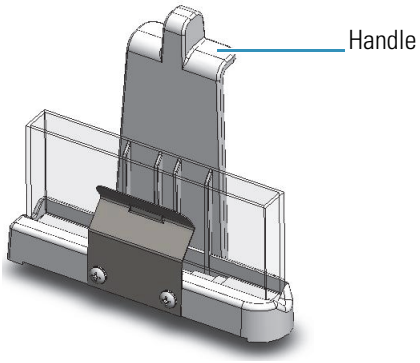
Installing a Manual Accessory

Installing a Reference Cell Holder

Installing a Reference Cell Holder

❖ **To install a reference cell holder**

1. Open the reference compartment cover. See Opening and Closing the Sample and Reference Compartment Doors.
2. Grasp the cell holder by the handle and lower it into the reference compartment.



3. Insert the pin in the alignment hole and press the holder in place.
4. Close the reference compartment cover.

For descriptions of the available reference holders, see *Reference Cell Holders* or search for that name in the INSIGHT Help system.

Related Topics

Installing a Smart Accessory

Installing a Manual Accessory

Installing a Liquid Thermostatted Accessory

Using the Spectrophotometer

This section explains how to set up a Computer Control or Local Control spectrophotometer and perform basic operations using the keypad.

Contents

Computer Control Instruments

Computer Control instruments must be connected to an external computer with the proper software installed.

All instrument features can be run from the computer using our INSIGHT software. Basic operations such as powering the spectrophotometer on and off, measuring a sample or baseline and running predefined macros can also be performed from the instrument keypad (see Keypad Operation).

Related Topics

Setting up a Computer Control Instrument

Turning off a Computer Control Instrument

Local Control Instruments

Setting up a Computer Control Instrument

❖ **To set up a Computer Control instrument**

1. Connect the supplied USB cable to the Computer port on the back of the spectrophotometer.
2. Connect the other end of the cable to the USB port on the back of the computer.
3. Turn on the spectrophotometer main power switch (on the back panel).
4. If the main power switch is already on, press the **Power** button on the keypad.

   Wait for the system to initialize. This may take several minutes. The power indicator on the keypad stops blinking when initialization is complete.

Note If the instrument fails to initialize, the power indicator blinks rapidly. Contact us.

5. Turn on the remote computer.
6. Double-click the INSIGHT desktop icon to start the software.

Tip For routine use, leave the power switch on the instrument back panel in the On position and use the **Power** button on the keypad to turn the instrument on and off (press and hold the keypad **Power** button **4 seconds** to power off).

Related Topics

Turning off a Computer Control Instrument

Local Control Instruments

Turning off a Computer Control Instrument

❖ **To turn off the instrument**

1. Close INSIGHT software.
2. Hold down the **Power** button on the keypad for **4 seconds**.
3. If necessary, turn off the spectrophotometer main power switch (on the back panel).

Related Topics

Setting up a Computer Control Instrument

Local Control Instruments

Local Control Instruments

Local Control instruments include a touchscreen and an embedded computer preloaded with INSIGHT software. Follow these steps to set up a Local Control instrument to run from the touchscreen or from a remote computer.

All instrument features can be run from the touchscreen or remote computer. Basic operations such as powering the spectrophotometer on and off, measuring a sample or baseline and running predefined macros can also be performed from the instrument keypad (see Keypad Operation).

Related Topics

Setting up a Local Control instrument for touchscreen operation

Setting up a Local Control instrument for remote operation

Turning off a Local Control Instrument

Computer Control Instruments

Setting up a Local Control instrument for touchscreen operation

❖ **To set up a Local Control instrument (no computer connected to the instrument)**

1. Turn on the spectrophotometer main power switch (on the back panel).
2. Press the **Power** button on the keypad.

   Wait for the system to initialize. The power indicator on the keypad stops blinking when initialization is complete. INSIGHT software starts automatically and appears on the touchscreen.

Tip For routine use, leave the power switch on the instrument back panel in the On position and use the **Power** button on the keypad to turn the instrument on and off.

Related Topics

Setting up a Local Control instrument for remote operation

Turning off a Local Control Instrument

Computer Control Instruments

Touchscreen keyboard

Setting up a Local Control instrument for remote operation

❖ **To set up a Local Control instrument to run from a remote computer**

1. Connect the supplied USB cable to the Computer port on the back of the spectrophotometer.
2. Connect the other end of the cable to the USB port on the back of the computer.
3. Turn on the spectrophotometer main power switch (on the back panel).
4. Press the **Power** button on the keypad.

   Wait for the system to initialize. The power indicator on the keypad stops blinking when initialization is complete. INSIGHT software starts automatically and appears on the touchscreen.
5. Make sure the remote computer is on.
6. Do one of the following:
   - Hold down the **Function 4** and **Function 2** keys on the instrument keypad for **4 seconds**.
   - From the INSIGHT software displayed on the touchscreen, choose **System Settings > System.** In the Instrument Control group, select **Computer.**
7. Double-click the **INSIGHT** desktop icon on the remote computer to start the software.

Note To return control to the touchscreen, from the INSIGHT software displayed on the remote computer:

1. Do one of the following:
   - Choose **System Settings > System.** In the Instrument Control group, select **Instrument Local**.
   - Hold down the **Function 4** and **Function 1** keys on the instrument keypad for **4 seconds**.
2. Double-click the **INSIGHT** desktop icon on the touchscreen to start the software.

Related Topics

Setting up a Local Control instrument for touchscreen operation

Turning off a Local Control Instrument

Computer Control Instruments

Turning off a Local Control Instrument

❖ **To turn off the instrument**

1. Close INSIGHT software.
2. Press the **Power** button on the keypad.
3. Respond to the prompt to shut down Windows software.
4. If necessary, turn off the spectrophotometer main power switch (on the back panel).

Tip For routine use, leave the power switch on the instrument back panel in the On position and use the **Power** button on the keypad to turn the instrument off.

Related Topics

Setting up a Local Control instrument for touchscreen operation

Setting up a Local Control instrument for remote operation

Computer Control Instruments

Keypad Operation

Use the keypad on the Evolution 200 instrument front panel to perform the following operations.

Button	Label	Function
	Enter	Confirm highlighted choices in menus and text entry boxes.
	Run	Perform the measurements specified using the current settings. When the Loading Guide is displayed, pressing Run is equivalent to clicking the OK button in the software.
	Stop	Stop the current operation. When the Loading Guide is displayed, pressing Stop is equivalent to clicking the Cancel button in the software.
	Zero/Base	Perform a zero measurement or baseline scan.
	Power	Turn the instrument on or off (press and hold 4 seconds to power off).
	Function	Start an assigned program or CUE script. See Assigning Programs to Function Keys for details. Use the CUE application to edit scripts. See the CUE on-line help for details.

Keypad Shortcuts

Action	Keypad Shortcut
For Local Control instruments:	
• Switch INSIGHT control from touchscreen to remote computer	Function 4/Function 2 (hold down 4 seconds)
• Switch INSIGHT control from remote computer to touchscreen	Function 4/Function 1 (hold down 4 seconds)

Assigning Programs to Function Keys

The function keys on the Evolution 200 instrument front panel are managed by the INSIGHT Launcher application, which opens automatically each time a user logs on to the system computer. When the INSIGHT Launcher is running, the INSIGHT icon appears in the Windows status tray area (on the right side of the Windows task bar) as shown below.

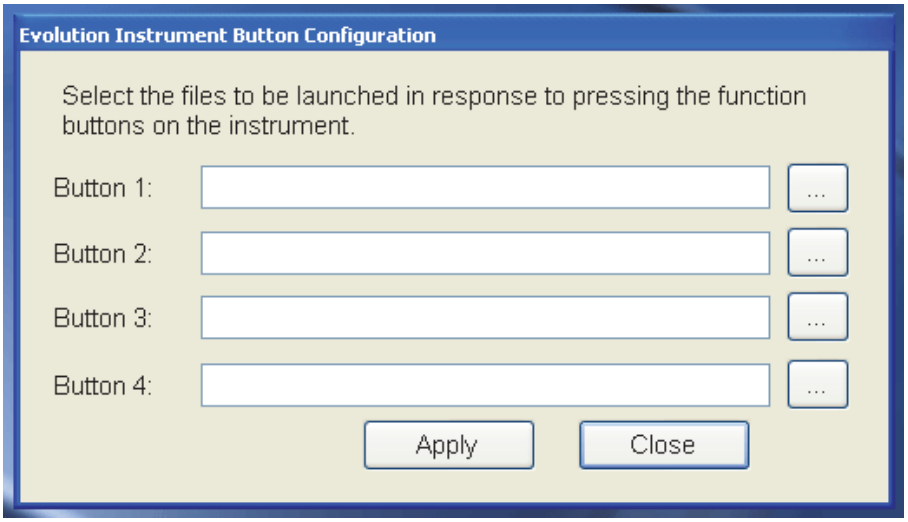
Each function key can be assigned to any executable program such as the Windows Notepad application or a script created using our CUE application.

Note Function key assignments are shared by all users of the system computer. However, a specific user's ability to run a program from a function key is controlled by their Windows user account privileges, which are assigned by the Windows administrator.

❖ To assign an executable program to a function key

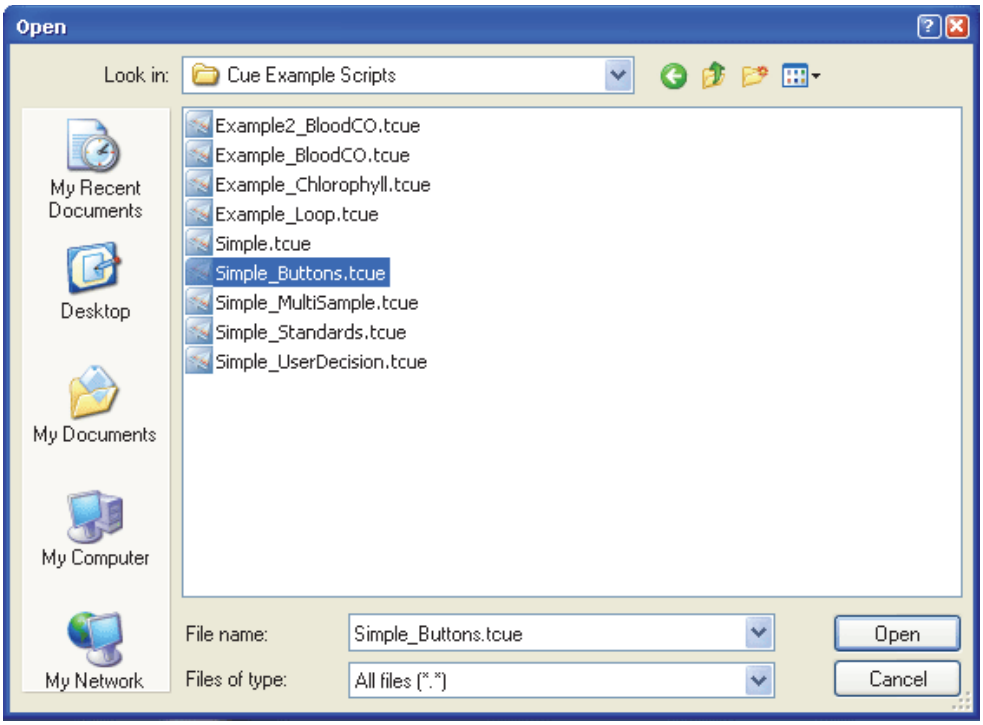
1. Open INSIGHT software.
2. Double-click the INSIGHT icon on the right side of the Windows task bar.

   The Instrument Button Configuration window is displayed.



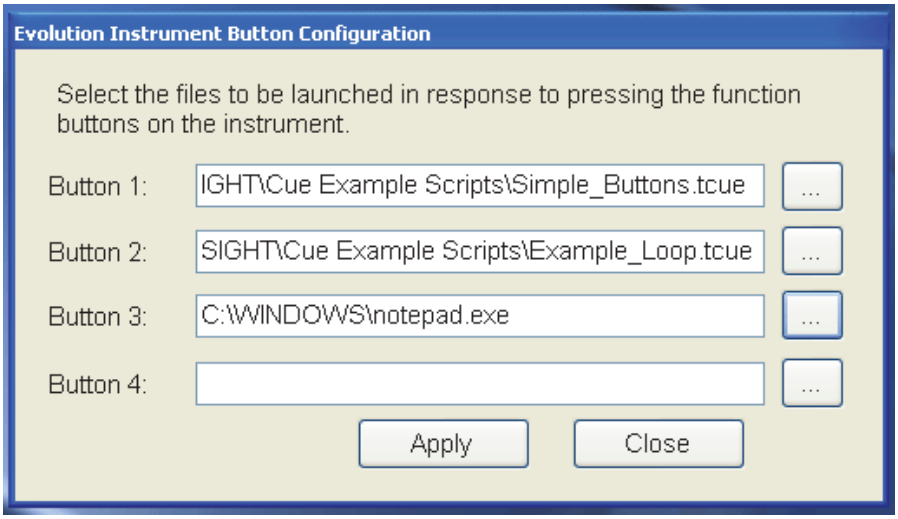
3. To associate an executable program with a function key, enter the program's full path name in one of the Button boxes or use the browse (...) button to the right of an entry box to locate and select the program and then click **Open**.

   This example shows how to select a script from the CUE Example Scripts folder provided with INSIGHT software. CUE scripts have the file name extension *.TCUE.



4. Assign other programs to the remaining function keys as desired.

   The example below shows two CUE scripts assigned to buttons 1 and 2 and the Windows Notepad application assigned to button 3.



5. When you are finished assigning programs to function keys, click **Apply** and then click **Close**.

   The button assignments are persistent and will be active the next time a user logs on.

Maintenance

Contents

Routine Maintenance

Clean the instrument regularly using approved methods (wipe off spilled chemicals immediately).

Related Topics

Maintenance

Cleaning the Instrument

Clean the exterior of the instrument and the sample and reference compartment interior periodically. These compartments have a port to allow small amounts of liquid to drain from the instrument.

NOTICE Do not allow moisture to leak into the instrument interior.

To clean the touchscreen, see Cleaning the Touchscreen.

❖ To clean the instrument exterior and the sample or reference compartment interior

1. Use a lint-free cloth dampened with a weak solution of detergent and water to wipe the surface as necessary.
2. Repeat using a cloth dampened with plain water.
3. Dry the surface with another cloth.

Related Topics

Maintenance

Cleaning the Touchscreen

Clean the instrument touchscreen as needed to remove dust, fingerprints and other materials.

❖ To clean the touchscreen

1. Press the **Power** button on the keypad to turn off the spectrophotometer.
2. Wipe the screen gently with a soft, lint-free cloth dampened (not wet) with distilled water.

   To remove stubborn materials or oil, use a standard screen cleaner kit that includes antistatic wipes. Spray a little solution on the wipe and rub it gently across the screen.
3. Dry the screen with a clean, lint-free cloth.

Related Topics

Maintenance

Removing and Installing the Detector Module

Some accessories have a dedicated detector and require removing the instrument detector module before they can be installed.

Avoid UV radiation hazard in the detector compartment. Protect eyes and skin from exposure.

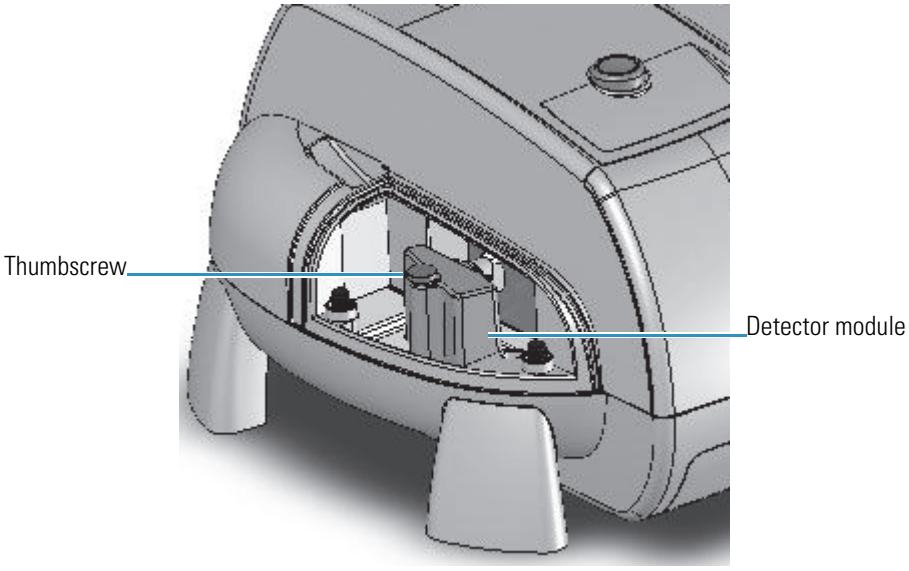
CAUTION Safety glasses with side shields or goggles with solid side pieces are the only equipment that provides adequate eye protection against direct and reflected UV light.

❖ To remove the detector module

1. Turn off the spectrophotometer main power switch (on the back panel).
2. Remove the detector access cover. See Detector Access Cover.

3. Loosen the thumbscrew that attaches the detector module to the instrument, and carefully lift the module straight up.

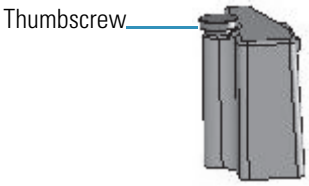
NOTICE Avoid touching the connector pins on the bottom of the detector module.



4. Replace the detector access cover.
5. Store the detector module in a secure, dust-free location.

❖ To install the detector module

1. Turn off the spectrophotometer. See Using the Spectrophotometer.
2. Remove the detector access cover. See Detector Access Cover.
3. Unplug the accessory detector cable if necessary.
4. Position the detector module so its thumbscrew faces left.



5. Line up the connector on the bottom of the detector module with the connector in the detector compartment, and then press down on the module to secure the connection.
6. Tighten the thumbscrew by hand.
7. Replace the detector access cover.

Related Topics

Maintenance

Detector Access Cover

Detector Access Panel

Ordering Parts

To order replacement parts, contact us using the information provided at the beginning of this document.

INSIGHT Software

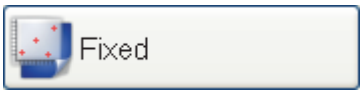
Contents

Applications

Contents

INSIGHT Bio Applications may also be available.

Fixed



The Fixed application measures the light passing through the sample at one or more wavelengths. To work with this application, click **Fixed** in the right pane in Home.

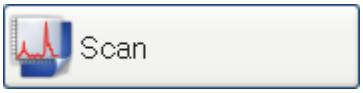
Related Topics

The INSIGHT Window

Performing a Fixed Measurement

Setting Application Parameters

Scan



The Scan application measures the light that passes through the sample over a range of wavelengths. To work with this application, click **Scan** in the right pane in Home.

Related Topics

The INSIGHT Window

Performing a Scan Measurement

Setting Application Parameters

Quant

Use the Quant application to set up and perform quantitative analyses of sample data for Fixed or Scan applications. To work with this application, click **Quant** in the right pane in Home.

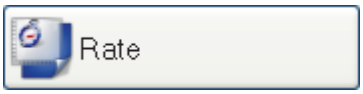
Related Topics

The INSIGHT Window

Setting Application Parameters

Performing a Quantitative Analysis

Rate



Use the Rate application to make time- and temperature-based kinetics measurements. To work with this application, click **Rate** in the right pane in Home.

Related Topics

The INSIGHT Window

Setting Application Parameters

Performing a Rate Measurement

Live Display

Use the Live Display application for quick measurements and simplified data collections in Fixed or Scan mode. To work with this application, click **Live Display** in the right pane in Home.

Related Topics

The INSIGHT Window

Acquiring Data using Live Display

Performing Fixed Measurements

Performing Scan Measurements

The INSIGHT Window

The INSIGHT window is divided into panes. At the left is the navigation pane, which contains task buttons and action buttons for displaying software features and performing operations. The right pane provides access to applications, stored data, software settings and data acquisition features.

Click **Home** in the navigation pane at any time to see applications available in the right pane.

Above the panes is a menu bar that can include features for working with workbooks, performing math, analysis and other operations, and getting help. The available features depend on the selected application, whether spectral data is displayed, etc.

To display a keyboard for entering information or for "right-clicking" software features (for example, to display a shortcut menu) on a Local Control system, click this icon in the lower-left corner of the INSIGHT window. See the procedure below for more information.

Alternatively, click this icon on the Windows taskbar. If the taskbar is not visible, click the bottom (or other) edge of the Windows desktop to display it.

❖ **To "right-click" a software feature on a Local Control system**

1. Click the feature.
2. Display the keyboard by clicking or .
3. Click the key indicated below.

Related Topics

Navigation Pane

Right Pane

Menus

Instrument Status Monitors

Navigation Pane

The navigation pane at the left side of the window contains features for the current application:

Task Buttons
Action Buttons
Other Navigation Pane Features

Related Topics

Right Pane

Menus

Task Buttons

The main task buttons (present when the software starts) provide access to basic operations:

Button	Description
Home	Gives access to user groups and their applications. (The default is Classic.)
My Data	Provides tools for storing and retrieving data and other information.
Options	Includes features for controlling how users interact with the software.
System Settings	Provides features for aligning accessories, calibrating lamps, updating firmware and performing other tasks. If a Local Control instrument is connected to an external computer running INSIGHT software, use the **Instrument Control** options on the System tab to select the desired location of instrument control.

Other task buttons may be available for specific applications:

Button	Description
Measure (current application)	Displays data acquisition features for the current application.
Reports	Configures reports containing sample data and specifies how to print reports.
Oligo Calculator	Calculates molecular weight, extinction coefficients, concentration factors and melting points for nucleic acid sequences.
Dye/Chrom. Editor	For entering and editing new dyes rather than using one of the precoded default dyes.

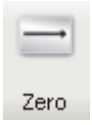
Related Topics

Action Buttons

Other Navigation Pane Features

Action Buttons

These action buttons appear at the top of the navigation pane when appropriate for the opened application:

Button	Description
	Starts sample data acquisition, displaying prompts for confirming samples information, loading samples, saving data or performing other actions. Available only after a blank measurement for some applications. May measure single or multiple samples, depending on the application settings.
	Measures the baseline for the application.
	Measures the blank for the application.
	Stops data acquisition.
	Prints a copy of the spectrum and associated sample data to the default printer.

Note To start data acquisition automatically after a specified delay instead of responding to a prompt, use Enable auto input trigger on the Preferences tab in Options.

Other action buttons may appear for some tasks.

Related Topics

Task Buttons

Other Navigation Pane Features

Other Navigation Pane Features

Feature	Description
Overlay data	Displays multiple spectra or multiple fixed data points as they are acquired. The overlaid sample IDs appear in the upper-left corner of the data display. To display the spectrum or data point of interest in red, click its sample ID. The last sample measured appears in red at the top of the legend. Available for Fixed and Scan applications only.
Advanced Calculations	For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate. To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**. ❖ **To add column and row headings to the calculations table** 1. Click a cell that needs a heading or other label. 2. In the Advanced Calculations Cell Properties box, choose **Text**. 3. Type the heading or label text. 4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**. 5. To display the cell contents in bold text, click **Bold Text**. 6. Click **OK**.

Feature	Description
	❖ **To define calculations based on the samples table** 1. In the calculations table, click the cell where you want the calculation result to appear. 2. In the Advanced Calculations Cell Properties box, choose **Calculation**. 3. Select an equation in the **Equation** list box. The list of available equations is fixed. 4. Set Source to **Samples table**. 5. Select a sample measurement in the **Column** list box. 6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box. Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5). 7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**. 8. To display the cell contents in bold text, click **Bold Text**. 9. Choose **OK**. The calculated result appears in the selected cell.

Feature	Description
	❖ **To define a calculation based on the calculations table** 1. In the calculations table, click the cell where you want the calculation result to appear. 2. In the Advanced Calculations Cell Properties box, choose **Calculation**. 3. Select an equation in the **Equation** list box. 4. Set Source to **Calculations Table**. 5. Specify the table cells to include by entering their ID numbers in the **Range/Reference** box. If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5). If Equation is set to **Addition**, **Subtraction**, **Multiplication** or **Division**, specify one cell location in each Reference box. (e.g., A1). If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor. 6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**. 7. To display the cell contents in bold text, click **Bold Text**. 8. Choose **OK**. The calculated result appears in the selected cell.

Related Topics

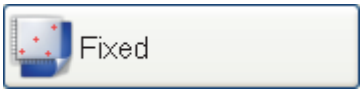
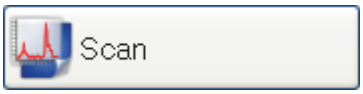
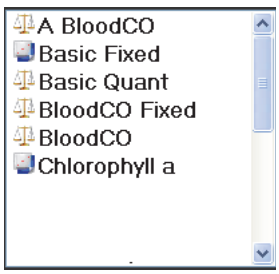
Adding Custom Calculations

Task Buttons

Action Buttons

Right Pane

The right pane in Home includes:

Feature	Description
	Select a user group to access its applications (see "Application buttons" below). See Configuring the Home Page for information on associating applications with user groups.
 	Click a button (for example, Fixed or Scan) to open an application. The available applications depend on the selected user group (see "Group" above).
	Click this button to access instrument performance tests. See Performance Verification.
	Click this button to access Live Display mode, which allows quick collection of data using the Fixed or Scan application. The data can be collected in absorbance or % transmittance. The results can be displayed, printed and stored.
	If this template list is present, you can double-click a template to open it and the associated application. See Configuring the Home Page for information on associating the Template list with user groups.

When the Home screen is not displayed, the right pane is used for application settings, sample measurement results, available workbooks, and other features.

Related Topics

Data Display

Working With Labels

Navigation Pane

Menus

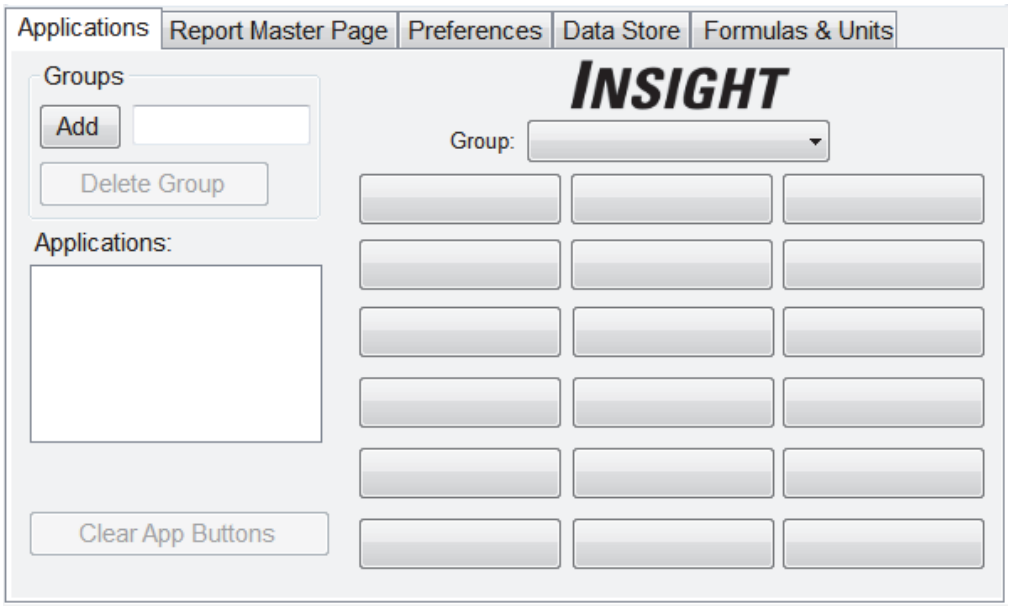
Configuring the Home Page

The Home page can be configured to display the application buttons and templates you use most frequently. You can group and arrange application buttons to suit one or many users. The template list can be displayed or hidden.

❖ To access the features for configuring the home page

1. Click **Options**.

2. Click the Applications Tab.



❖ To add a new group

Enter the desired group name in the **Groups** box and click **Add**.

After you click Add, the new group name appears in the Group box and the available applications appear in the Applications box.

❖ To customize the current group

Drag the desired applications from the **Applications box** to the buttons at the right. To remove an application from the group, drag it from its button to the Applications box.

❖ To delete the current group

Click **Delete Group** and then click **Yes** when prompted.

❖ **To reset the application buttons for the current group**

Click **Clear App Buttons** and then click **Yes** when prompted.

❖ **To make custom templates available for groups other than Classic**

Drag the List of Templates item from the **Applications box** to one of the top nine menu buttons.

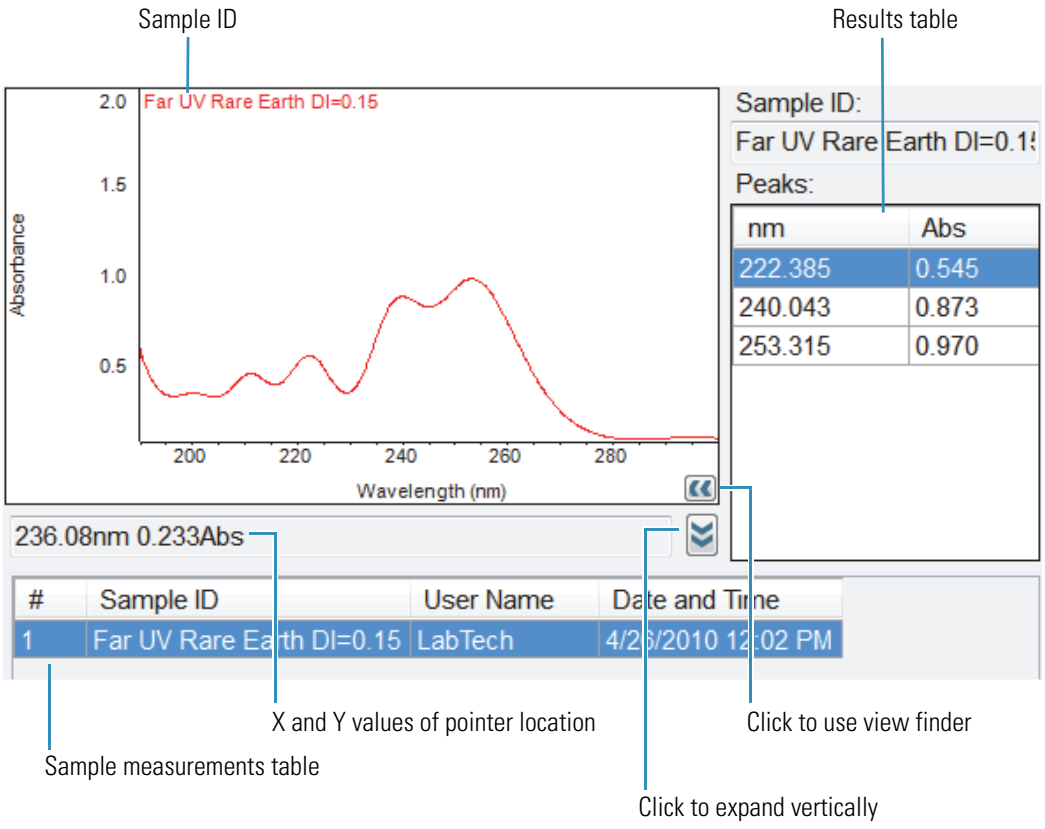
Related Topics

Applications Tab

Right Pane

Data Display

For some applications, clicking the Measure task button provides a data display in the right pane for viewing sample data immediately after acquisition or from previous measurements.



All data acquired with the current workbook are listed in the sample measurements table. To view data, select them in the list. To select multiple spectra (or sample points), hold down **Shift** or **Ctrl**. To display data from multiple samples as they are measured, select **Overlay data** in the navigation pane.

The sample IDs appear at the top of the data display, with the selected spectrum (or sample points) and sample ID in red. To turn off the sample IDs, right-click the data display, point to **Sample Legend** and choose **OFF**.

For scan data, the X and Y values of the pointer location appear below the data display. To enlarge a region, draw a box around it and click inside the box. Alternatively, click  (if available) to reveal the view finder which has additional display tools.

To expand the data display vertically, click  .

To change the appearance of the axes or data, right-click an axis and choose **Display Attributes**. View the effects on the display while adjusting the attributes. To change the color of the axes or their labels, click the appropriate color box. Your selections will affect the appearance of data on the display and in printed reports.

The **Sample ID readout** at the right of the display shows the name of the sample being measured (or to be measured). The **results table** below the Sample ID shows the results of analysis operations (Peak Pick and Value Level Crossings for Scan data) and whether sample points fall within specified control limits (for Fixed data).

Right-click the display to access these features (if available for the current data type):

Feature	Description
Copy To Clipboard	Copies the plotted data as it is currently displayed to the Windows® Clipboard.
Autoscale	Adjusts the vertical scale of each spectrum for optimal viewing. Double-clicking the display has the same effect.
Set Scale	For setting minimum and maximum values for the axes. To set them automatically, select **Autoscale**.
Auto Arrange Labels	Positions labels for easier reading.
Sample Legend	Specifies whether to display sample IDs near the upper-left corner of the pane.
Sample Labels	Specifies whether to display spectra with annotations (see "Annotate" below) and labels, such as those showing absorbance values for specific wavelengths.
Line Type	Specifies whether to display scan spectra using solid lines or individual data points.

Feature	Description
Annotate	Provides features for adding annotation to displayed data. (Added annotation is displayed only for the selected data.) See Working With Labels for information about manipulating added labels. Right-click an edge of an added ellipse, rectangle or image to access ways to manipulate or delete the object. The availability of features depends on the current application. More: **Attach to Curve** displays the X value of the pointer location as a label attached to a scan spectrum by a line. **Free Standing Text** adds an editable label at the pointer location. Its position is not affected by zooming in and out. **Ellipse** draws an ellipse. To move it, drag its top edge. To resize it, drag a side or bottom edge. Its position, size and shape are affected by zooming in and out. **Rectangle** draws a rectangle. To move it, drag its top edge. To resize it, drag a side or bottom edge or a corner. Its position, size and shape are affected by zooming in and out. **Image** displays a dialog box for locating and selecting an image to add to the display. To move the image, drag its top edge. To resize it, drag the bottom edge or a corner. Its position and size are affected by zooming in and out. **Floating Image** works the same as Image (see above), except the image position and size are not affected by zooming. **Tool Value** displays the measurement made by the Peak/valley measurement tool or peak area tool as a label attached to the spectrum by a line.

Feature	Description
3D Display (Scan Rate only)	Shows the selected scan data in a three-dimensional surface plot with these axes: absorbance, wavelength and time since the start of the experiment (vertical axis). You can rotate the image, zoom in or out, or drag the image within the pane. More: **Rotate**. To rotate the image, **drag an edge or corner** as though you were swinging that point around the center of the image. If you release the mouse button while dragging the point, the image continues to move in an "animated rotation." To stop this rotation, click the image. **Zoom**. To enlarge or shrink the image, **hold down the right mouse button and drag vertically** across the image. You can also enlarge or shrink the image by clicking it and turning the mouse wheel (if present). **Pan**. **Hold down the Shift key and drag the image** in any direction to move it within the pane. When you click a point in the 3-D image, the spectral display pane changes to show the spectrum collected at the clicked time value. To display multiple spectra, hold down the Shift key while clicking locations in the 3-D image. To adjust the display limits of the 3-D image, draw a box around an area of interest in the spectral pane and click inside the box. To redisplay the full data range and adjust the vertical scale to fill the vertical axis, right-click the spectral display pane and choose **Autoscale**. To remove the 3-D image from the window, right-click the spectral display pane and choose **3D Display** to clear the check mark or choose **View > 3D Display**.

Feature	Description
More Display Options (Fixed only)	Specifies how to display fixed sample data and control limits: **Connect points** (in Spectrum) draws connecting lines between sample points. **Show annotations** displays X and Y values of sample points. **Show X grid** draws light vertical lines through sample points, making it easier to see their X values. **Show Y grid** draws light horizontal lines through sample points, making it easier to see their Y values. If Use control limits is selected on the Instrument Tab for Fixed, **Show limit lines** displays the control limit lines for each measured wavelength.

Related Topics

Working With Labels

Right Pane

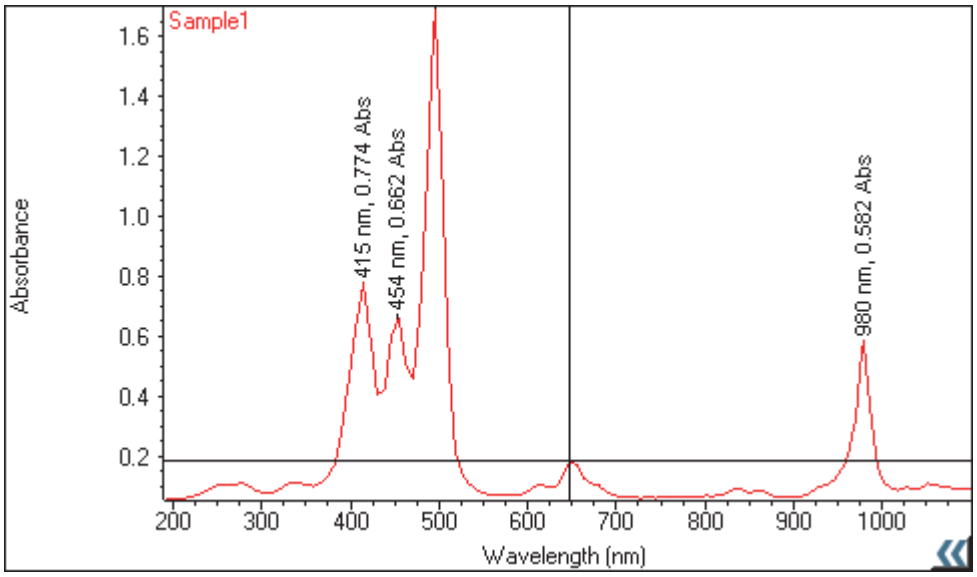
Measuring Samples

Measurement Results

The INSIGHT Window

Working With Labels

Some operations place labels on the displayed data. Here is an example:



❖ To show or hide labels

Right-click the pane, point to **Sample Labels** and choose **ON** or **OFF**.

❖ To move a label

Drag it to the new position.

❖ To edit a label

Double-click it and use the dialog box that appears to change the text, its font or its color, or right-click the label and choose **Edit**, **Color** or **Font**.

❖ To rotate a label

Right-click it, point to **Rotate** and choose an orientation.

❖ To move a label in front of or behind annotation

Right-click it and choose **Send to Front** or **Send to Back**. Click here for information about adding annotation.

❖ To delete a label

Right-click it and choose **Delete**.

Related Topics

Using the Palette Tools and View Finder

Finding Peaks in Scan Data

Finding Value Level Crossings in Scan Data

Data Display

Measuring Samples

If the current application provides a Measure button in the navigation pane, use it to access data acquisition features for the application. The name of the application appears on the button; for example:

Clicking the button displays action buttons for starting sample measurement or other data acquisition. The provided buttons and their availability depend on the current application and whether necessary operations have been performed. See Action Buttons.

Measurement results appear in the right pane.

See Setting Application Parameters for information about setting parameters that affect data acquisition, including those for controlling sampling accessories. For information about sampling accessories for the Evolution 200 Series instruments, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

Related Topics

Performing Fixed Measurements

Performing Scan Measurements

Performing a Quantitative Analysis

Performing Rate Measurements

INSIGHT Bio Applications

Measurement Results

Below the data display in some applications is a table of sample measurement results. To display a spectrum, click its row in the table.

To print a report containing results information for a sample, select it in the table and click **Print** or choose **File** (menu) **> Print Report**. To preview the report, choose **File > Print Preview.** To specify the information to include, use the **Print** tab and **Reportable Data** tab in Reports.

To view, copy or print information about a spectrum, including its history, right-click it in the table and choose **Properties**.

To measure a sample again, right-click its row in the sample measurements table and choose **Remeasure** (if available). (If Remeasure is not available, you can choose **Options > Preferences** and clear **Prevent removal of data** to enable it.) After the remeasurement, the previous information for the sample is either crossed out or removed from the table, depending on the status of **Prevent removal of data** on the Preferences tab in Options.

To delete a spectrum from the workbook, right-click it in the table and choose **Remove** (if available). To delete all the spectra from the workbook, right-click the table and choose **Remove All** (if available).

To append data to a workbook, click **Measure**.

Any Peak Pick or Value Level results appear in the results table to the right of the data display.

Menus

The File menu, when available, includes:

Command	Description
New Workbook	Opens a new workbook and closes and saves the current workbook.
Merge Workbooks	If available, allows you to copy data between workbooks created with the same application. Instructions: 3. Open a workbook that was created using the desired application such as Fixed or Scan. See My Data. 4. Choose **File > Merge Workbooks**. 5. In Merge Workbooks, use the navigation (left) pane to locate the workbook that contains the data you want to copy. A list of workbooks created using the selected application appears in the Workbooks pane. If the workbook contains sample data, the sample names are listed in the Samples pane. The data associated with the selected sample are displayed in the Data pane. 6. Select the sample measurements to import by clicking their names in the Samples pane. To select multiple samples, hold down **Shift** or **Ctrl**. 7. Click **OK**. 8. The sample names and other information are appended to the sample measurements table in the open workbook. 9. Choose **Save Workbook** or **Save As Workbook** to save the new data to the current or another workbook. The merged workbook is unchanged.
Save Workbook	If available, saves the current workbook using its current file name and location.
Save As Workbook	If available, saves the current workbook with the specified file name and location.
Close Workbook and Go Home	Closes and saves the current workbook and returns to Home.
Close All Workbooks and Go Home	Closes all open workbooks (all applications) and returns to Home.

Command	Description
Import	If available, imports an externally collected spectrum (.csv file or .tsv file) into the current Scan workbook.
Print Preview	Shows the current report before printing, with the header and footer specified on the Report Master Page tab in Options.
Print Report	Prints the current report to the default printer.
Use Current Settings as Default	Makes the current workbook settings and report configuration selections the default choices for a newly opened workbook for the application.

Save Workbook Settings as Template	If available, saves the current software settings as a template that can be used later by the specified user group.
	
	More
	
	To share a custom template, paste a copy of it into the desired folder, such as:
	
	`C:\Users\Public\Documents\Thermo\Insight\Custom Methods`
	
	`C:\Documents and Settings\All Users\Documents\Thermo\Insight\Custom Methods`
	
	Note: "Shared Documents" may appear in a path instead of "Documents.

E-mail Current Workbook	Automatically attaches the current workbook to a new e-mail message.

The View menu includes:

Command	Description
3D Display (Scan Rate only)	Shows scanned rate data in a three-dimensional surface plot.

The Math menu, when available, includes:

Command	Description
Smooth	Reduces noise in wavelength scan data from Scan and Rate experiments. The smoothing algorithm uses the Savitzky-Golay method.
Derivative	Converts scan data from Scan and Rate experiments to a derivative.
Convert Spectra	Converts wavelength scan data to another Y-axis format.
Add	Adds two scan spectra.
Normalize	Adjusts the Y scale of scan data so that a selected data point has the desired Y value.

Command	Description
Subtract	Subtracts one scan spectrum from another.
Ratio	Divides one scan spectrum by another.
Average	Averages two or more scan spectra.
Factor	Adds a constant to scan data, subtracts a constant, multiplies by a constant or divides by a constant.

The Analyze menu, when available, may include:

Command	Description
Peak Pick	Finds peaks, valleys, or maximum and minimum values in a spectrum or region.
Value Level	Finds the wavelengths where a spectrum crosses a specified ordinate value.
Modify Rate Curve	Perform rate calculations or modify existing rate calculations on the data in a plot.

The Help menu includes:

Command	Description
Help	Displays the INSIGHT Help system, which can also be accessed from many software screens by pressing F1.
About	Provides information about the software version and instrument type.

Related Topics

Navigation Pane

Right Pane

Instrument Status Monitors

Various instrument status monitors may appear above the data display. They show information such as the current wavelength or readouts from installed accessories. You can double-click some status monitors to set operation parameters such as channel assignment. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

To specify the items to monitor, click this icon.

 If the system is operating normally, a green check mark appears on the Instrument Status icon at the bottom of the INSIGHT window.

 A yellow exclamation mark appears briefly on the icon when the system is busy performing an operation.

 If a system problem occurs, a red X may appear on the icon. Click it to see information about the problem.

Related Topics

Evolution 200 Series Accessories

Accessories Tab for Fixed

Accessories Tab for Scan

Accessories Tab for Quant

Accessories Tab for Rate

Thermo Software IQ

Thermo Software IQ performs Installation Qualification (IQ) for the software. IQ verifies that the correct software files were installed and can also be used to verify that these files have not been changed, deleted, or overwritten since they were installed. (For more information, see the Thermo Software IQ user guide available through the Help menu of the software.)

❖ **To start Thermo Software IQ**

1. Click **Start** on the Windows taskbar.
2. Choose **All Programs** (or Programs) **> Thermo > Thermo Software IQ**.

Contact Information

For help with questions or problems with the software, use the information at the beginning of this document to contact us.

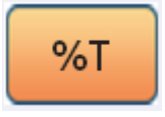
Operation

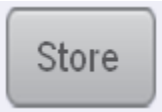
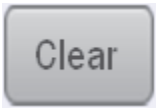
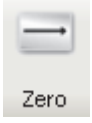
Contents

Acquiring Data using Live Display

The Live Display button appears on the Home page in both views (Classic and Bio Applications). Use Live Display for quick measurements and simplified data collections in Fixed or Scan mode. The data can be displayed in absorbance or % transmittance units. The results can be stored in a temporary location and printed on demand.

These features are available in Live Display:

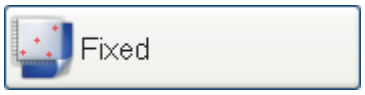
Feature	Description
	Selects the absorbance display mode. Data currently displayed in % transmittance are changed to absorbance.
	Selects the % transmittance display mode. Data currently displayed in % transmittance are changed to absorbance.

Feature	Description
	Selects the single wavelength data acquisition mode for the next measurement. Use the right and left arrows in the left pane to select the wavelength. The measurement results appear in the right pane.
	Selects the full scan data acquisition mode for the next measurement. Use the top set of right and left arrows in the left pane to select the starting wavelength for the scan; use the bottom set of arrows to select the ending wavelength.
	Records the currently displayed measurement in the computer's internal memory. Data from subsequent measurements are appended. To save the data, choose Print. The memory location is cleared automatically when you exit Live Display (or choose Clear). Pressing **Store** while in Fixed mode stores the measurement result along with the wavelength used and the display mode settings (Abs or %T). Pressing **Store** while in Scan mode stores a snapshot of the spectrum. If the spectral cursor tool is selected, it also stores the current X and Y coordinates of the cross hair.
	Clears all information from Live Display's associated memory location for storing analysis results. See Store.
	Starts sample data acquisition with Live Display. Available only after a blank measurement. Pressing **Measure** while in Fixed mode collects the data at the selected wavelength using a bandwidth of 1 nm and an integration time of 0.5 sec. After you press Measure, the Measure button is disabled and the software automatically takes a measurement every 2 seconds until you press Stop or Scan or exit Live Display (or after 5 minutes of inactivity). Pressing **Measure** while in Scan mode collects data at all wavelengths between the designated starting and ending wavelength using a bandwidth of 1 nm, an integration time of 0.05 sec, and a data interval of 1.00 nm. The selection and spectral cursor tools are available in Scan mode.
	Measures the blank for the selected application (Fixed or Scan).

Feature	Description
	Stops data acquisition.
	Prints a copy of the information from Live Display's associated temporary results file using the default printer. Available whenever stored measurements exist. See Store.
	The **selection tool** is available in Scan mode only. Use it to zoom in the spectrum. To zoom in, draw a box and click inside it. (Double-click anywhere in the data display to zoom out.)
	The **spectral cursor tool** is available in Scan mode only. Use it to view the X and Y values of a point in a spectrum. To view the X and Y values of a point, select the spectral cursor tool and click the data display. Cross hairs appear. The X and Y coordinates of their intersection with the spectrum appear below the data pane. To move the cross hairs, drag across the pane or use the left and right arrow keys on the keyboard.

Performing Fixed Measurements

Fixed measurements are based on the selected workbook. Measurements can be appended to an opened workbook or saved in a new workbook.

	Click **Fixed** in the right pane in Home to set up and perform a measurement at one or more specified wavelengths using the currently selected workbook or template. The tasks buttons below become available.
	To set data acquisition parameters for the current Fixed workbook, click Settings.
	To access features for performing the measurement, click Measure Fixed.
	To select a different workbook or create a new one for the Fixed application, click My Data or choose **File** < New Workbook.

Note Change Fixed workbook settings as desired before making sample measurements. All Fixed measurements are saved in a workbook that includes the data acquisition settings. See Setting Application Parameters for information about unlocking settings.

Related Topics

Fixed

Performing a Fixed Measurement

Performing a Fixed Measurement

❖ To perform a fixed measurement

1. Select or create a workbook.
   - To append new data to an existing workbook, from the Home screen click **My Data**, click the **Workbooks** tab and double-click the workbook file.
   - To create a new workbook using the current template, from the Home screen click **Fixed** and then choose **File** (menu) > **New Workbook**.
   - To create a new workbook using a different template, from the Home screen click **My Data**, click the **Templates** tab and double-click a Fixed template file.
1. Click **Settings.**

2. Click the tabs in order to view or change the Fixed settings.

Note If you opened an existing workbook in step 1 above, the settings will be unavailable unless you unlock them. See Setting Application Parameters for information about unlocking settings.

3. Click **Measure Fixed**.

4. Load the sample to use to measure the zero or blank.
5. Click **Zero**.

6. Click **Measure**.

7. Follow the instructions that appear.
8. When samples information appears, modify it if desired.

More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

9. If only one sample will be measured, install it.
10. Click **Continue**.
11. Follow any instructions that appear, such as to install a specified sample.

The Data Display shows the acquired data (a fixed data point) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation.)

The table contains the columns of information specified on the Reportable Data tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their average values.

To measure a sample again, right-click its row in the sample measurements table and choose **Remeasure** (if available). (If Remeasure is not available, you can choose **Options > Preferences** and clear **Prevent removal of data** to enable it.) After the remeasurement, the previous information for the sample is either crossed out or removed from the table, depending on the status of **Prevent removal of data** on the Preferences tab in Options.

Related Topics

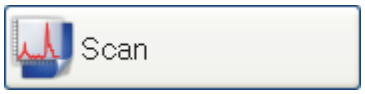
Fixed

Performing Fixed Measurements

Data Display

Performing Scan Measurements

Scan measurements can be appended to an opened workbook or saved in a new workbook.

	Click **Scan** in the right pane in Home to set up and perform a measurement over a range of wavelengths. The tasks buttons below become available.
	To set data acquisition parameters for the next Scan measurement, click Settings.
	To access features for performing the measurement, click Measure Scan.
	To select a different workbook or create a new one for the Scan application, click My Data or choose **File** (menu) < **New Workbook**.

Related Topics

Scan

Performing a Scan Measurement

Performing a Scan Measurement

❖ **To perform a scan measurement**

1. From the Home screen, click **Scan** > **Settings.**

2. Click the tabs in order to view or change the Scan settings.

   Note If you need to select a new workbook or template, click **My Data**, double-click a workbook or template, and then click **Settings**.

3. Click **Measure Scan**.

4. Load the sample to use to measure the baseline.

5. Click **Baseline**.

6. Click **Measure**.

7. Follow the instructions that appear.
8. When samples information appears, modify it if desired.

More:

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

9. If only one sample will be measured, install it.
10. Click **Continue**.
11. Follow any instructions that appear, such as to install a specified sample.

The Data Display shows the acquired data (a scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation.)

The table contains the columns of information specified on the Reportable Data tab in Reports. Examples include sample identification, user name, date and time, and integration time.

To measure a sample again, right-click its row in the sample measurements table and choose **Remeasure** (if available). (If Remeasure is not available, you can choose **Options > Preferences** and clear **Prevent removal of data** to enable it.) After the remeasurement, the previous information for the sample is either crossed out or removed from the table, depending on the status of **Prevent removal of data** on the Preferences tab in Options.

Related Topics

Scan

Performing Scan Measurements

Data Display

Performing a Quantitative Analysis

Click **Quant** in the right pane in Home to set up and perform quantitative analyses of sample data. The tasks buttons below become available.

To set Quant parameters, click **Settings**.

To access features for quantifying sample data, click **Measure Quant**.

To specify the predefined formulas, units and advanced equations to be available, click **Options** and use the Formulas & Units tab.

Related Topics

Settings for Quant Applications

Performing Quantitative Measurements

Formulas and Units Tab

Performing Quantitative Measurements

Contents

Related Topics

Performing a Quantitative Analysis

Data Display

Quantifying Samples Without Using Standards

Note Select **Manually entered factor** on the Type tab before using this procedure.

❖ **To quantify samples without using standards**

1. Click **Measure Quant** in Quant.

Note To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. Load the sample to use to measure the blank.
3. Click **Blank**.
4. Click **Measure**.

5. Follow the instructions that appear.
6. When samples information appears, modify it if desired.

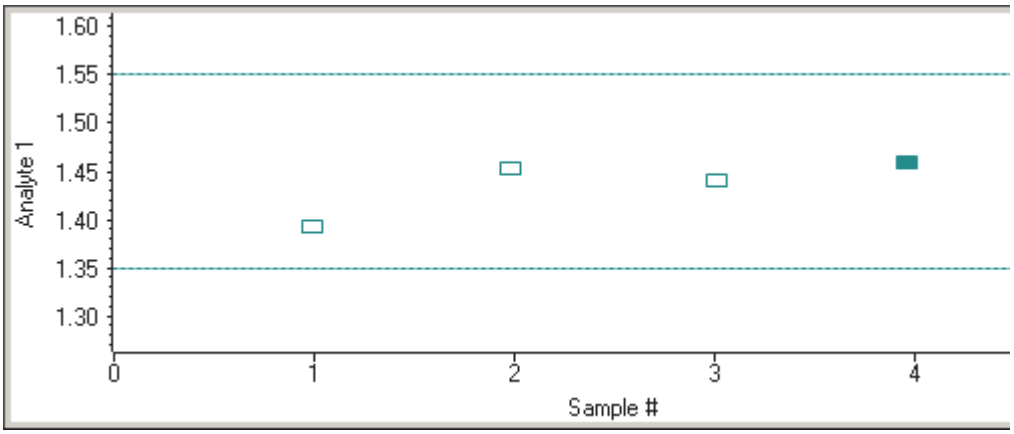
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, "D" at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, "D" and "T" at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

7. If only one sample will be measured, install it.
8. Click **Continue**.
9. Follow any instructions that appear, such as to install a specified sample.

The Run Chart tab plots the concentration of the measured component versus sample number. If **Use control limits** was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation.) See Data Display for more information.

The table contains the columns of information specified on the Reportable Data tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To measure a sample again, right-click its row in the sample measurements table and choose **Remeasure** (if available). (If Remeasure is not available, you can choose **Options > Preferences** and clear **Prevent removal of data** to enable it.) After the remeasurement, the previous information for the sample is either crossed out or removed from the table, depending on the status of **Prevent removal of data** on the Preferences tab in Options.

Related Topics

Quantifying Samples Using Standards

Performing a Quantitative Analysis

Data Display

Settings for Quant Applications

Formulas and Units Tab

Quantifying Samples Using Standards

Note Select **Measure single standard**, **Standard curve**, **Standard curve with two wavelengths** or **Advanced standard curve** on the Type tab before using this procedure.

❖ To quantify samples using standards

1. Click **Measure Quant** in Quant.

Note To start an analysis immediately after viewing or changing the template settings, start with the next step.

2. If standards need to be acquired, click **Measure**. If standards have already been acquired, skip to step 7 to acquire sample data.

3. Follow the instructions that appear. The instructions that appear are dependant on the current settings for the features on the Standards tab in Settings.

4. When standards information appears, modify it if desired.

More:

If Standard averaging on the Standards tab in Settings was set to Duplicate, "D" at the end of a standard name in the Confirm Standards List box indicates the second measurement to be made of the standard. If Sample averaging was set to Triplicate, "D" and "T" at the end of standard names indicate the second and third measurements to be made, respectively.

To enter previously saved standards information, use **Import Standards**.

If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each standard in the table.

To remove a standard from the list, select it, right-click the table and choose **Clear Standard**. To remove all the standards, choose **Clear Table**.

To save standards information for later use, use **Export Standards**.

5. Click **Continue**.
6. Follow the instructions that appear, installing the specified standards.

The Standard Curve(s) tab displays the resulting standard curve (or curves). Specify the standards to use for the curve (or curves) by selecting **Yes** or **No** in the **Use** column in the table. These selections can always be changed. After each change, the standard curve is updated automatically.

More:

If a standard curve is not calibrated, try...

- Selecting a different curve fit type.
- Remeasuring a standard using the correct standard material (select the listed standard, right-click the table and choose **Remeasure)**.
- Changing the setting of **Minimum r^2** on the Standards tab and acquire the standards again in a new workbook.

The table contains the columns of information specified on the Reportable Data tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To permanently remove an acquired standard from the analysis, right-click it and choose **Remove**. Its information is crossed out but not removed from the table.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the standard selected in the table. To access commands for customizing the display, including adding annotation, right-click the data. See Data Display for more information.

Note After a calibration curve is used to measure a sample, the curve can no longer be edited. However, you can open a new workbook based on the existing calibration data and then edit the curve before you begin acquiring new sample data.

7. Click **Measure**.

8. Follow the instructions that appear. The instructions that appear are dependant on the current settings for the features on the Samples tab in Settings.
9. When samples information appears, modify it if desired.

More:

If **Sample averaging** on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name in the Confirm Sample List box indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

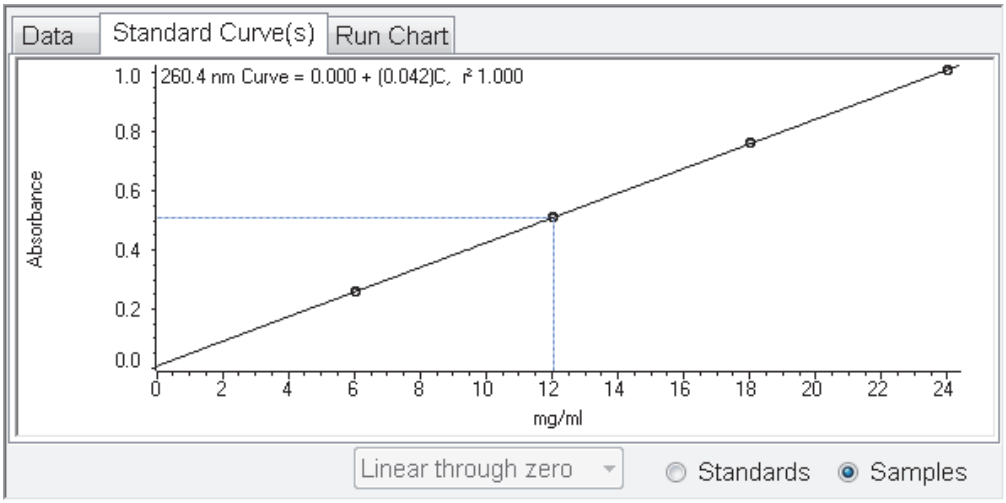
To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

10. If only one sample will be measured, install it.
11. Click **Continue**.
12. Follow any instructions that appear, such as to install a specified sample.

The sample measurements table contains the columns of information specified on the Reportable Data tab in Reports. If Sample averaging was set to Duplicate, “D” at the end of a sample name indicates the second measurement. If Sample averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

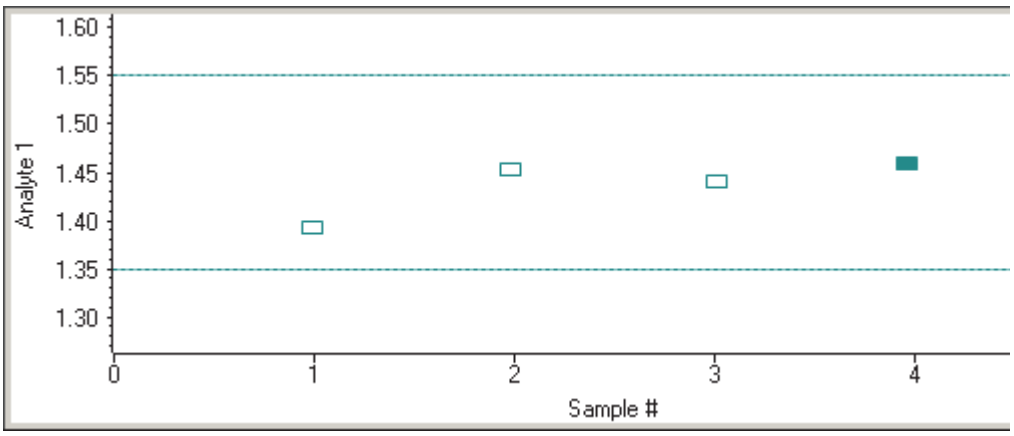
The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard

curve. A vertical line connects that point to the sample concentration value on the X-axis. The information at the top shows the measured wavelength, the equation for the standard curve and the calculated r^2 value, which indicates how well the standard curve fits the standard data points (1.0 is a perfect fit).



Note If there are two analysis wavelengths, the lines appear for each of the two standard curves.

The Run Chart tab plots the concentration of the measured component versus sample number. (If there are two analysis wavelengths, the concentration value is the average of the values for those wavelengths.) If Use control limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To measure a sample again, right-click its row in the sample measurements table and choose **Remeasure** (if available). (If Remeasure is not available, you can choose **Options > Preferences** and clear **Prevent removal of data** to enable it.) After the remeasurement, the previous information for the sample is either crossed out or removed from the table, depending on the status of **Prevent removal of data** on the Preferences tab in Options.

To copy the data on the Standard Curve(s) tab or Run Chart tab, right-click the plot and choose **Copy to Clipboard**.

Related Topics

Quantifying Samples Without Using Standards

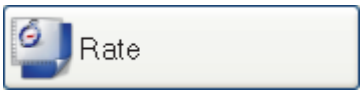
Performing a Quantitative Analysis

Data Display

Settings for Quant Applications

Formulas and Units Tab

Performing a Rate Measurement



Click **Rate** in the right pane in Home to set up and perform a kinetics measurement. The tasks buttons below become available.

To set Rate parameters, click **Settings**.

To access features for performing the measurement, click **Measure Rate**.

Performing Rate Measurements

Contents

Related Topics

Performing a Fixed Rate Measurement

Performing a Scanning Rate Measurement

Modifying a Rate Curve

Data Display

Performing a Fixed Rate Measurement

Rate measurements can be made at one wavelength or at up to 40 wavelengths in the same experiment. This includes multicell measurements, which allows running a fixed-wavelength measurement on several samples simultaneously using a sample changer.

Measurements are taken at specified intervals of time. Temperature-based measurements also record temperature and control the temperature setting and ramp rate for the duration of the experiment. Multiple stages can be programmed for time and temperature-based measurements.

The Time Status icon automatically appears above the data display during rate measurements.

❖ To perform a fixed rate measurement

1. Make sure Single wavelength or Multiple wavelength is selected on the Type tab in Settings in Rate.
2. Click **Measure Rate**.

3. Load the sample to use to measure the zero or blank.
4. Click **Zero**.

5. Click **Measure**.

   If necessary, the Zero action button can be used to remeasure the zero sample.

6. When samples information appears, modify it if desired.

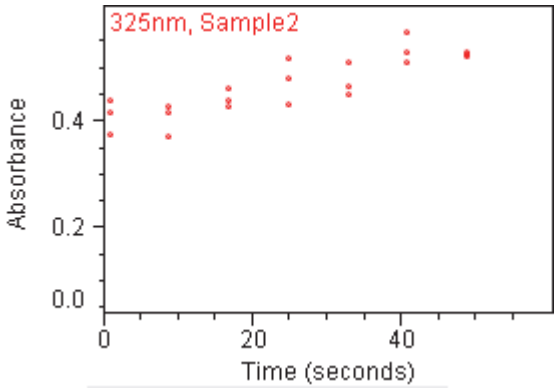
   To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

   Enter values in any special columns that were specified on the Samples tab in Settings. These values may appear in the rate data report.

7. Click **Continue**.

8. Follow the instructions that appear.

   Each row in the sample measurements table on the Data tab shows the measurement results for one sample measured at a specified wavelength. Each plot on the tab shows a sample's spectral intensity measured at a particular wavelength over time.



   To extend the collection time for the current sample measurement (without interrupting the data collection), click **Extend Time** and enter the amount of time to extend the measurement. If you are collecting data in multiple stages, only the current stage is affected.

   Scroll bars are provided if plots are out of view.

   Right-click the **Data tab** to access features for changing the scale or adding annotation. See Data Display for more information.

   The sample measurements table contains the columns of information specified in the Reported Columns pane of the Reportable Data tab in Reports.

9. To perform rate calculations or modify existing rate calculations on the data in a plot, **double-click it** or select **Analyze** (menu) < Modify Rate Curve.

   Note The features to set up rate calculations are available for time-based single and multiple wavelength rate measurements only. To calculate rate curves for temperature-based data (temperature and time are recorded), display the data with time on the X-axis and then **double-click the data display** or select **Analyze** (menu) < **Modifying a Rate Curve**.

Modifying a Rate Curve

Time-based single and multiple wavelength rate measurements can include a rate curve. To specify rate calculations, set up a fixed rate experiment and then select the Rate Calculations tab in Settings.

If **Calculate rate constants automatically at end of data acquisition** is selected on the Rate Calculations tab, after data collection completes, the rate calculations will appear in the sample measurements table along with the other sample data. To perform rate calculations or modify existing rate calculations on the data in a plot, double-click it or select **Analyze** (menu) > **Modify Rate Curve**. A window displays the plot and a table containing the default rate calculation values based on the Rate Calculations tab.

Note To calculate rate curves for temperature-based data (temperature and time are recorded), display the data with time on the X-axis and then **double-click the data display** or select **Analyze** (menu) < **Modifying a Rate Curve.**

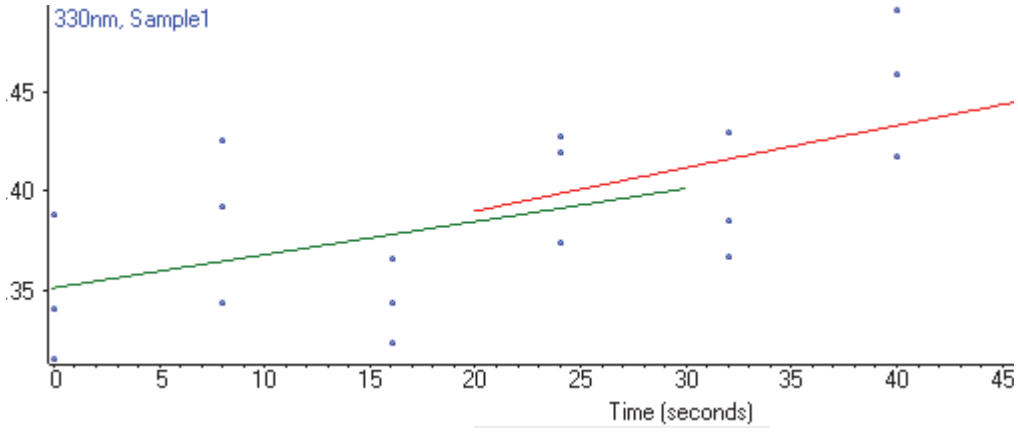
These operations are available from the Modify Rate Curve window.

- If desired, add or change sets of rate calculation values for the displayed data. Each set represents a curve that describes the rate behavior over the specified time period.

  To delete a row from the table, right-click it and choose **Delete Selected Row**. To delete all the information from the table, right-click it and choose **Clear Table**.

- If desired, use the vertical markers on the rate curve to adjust the data range used for rate calculations.

- Click **Update** to generate kinetic rate models, each of which appears as a line across the data.



Specify how to apply the lines and then click **Accept**.

This option...	Applies the lines to...
Selected plot only	The selected data only.
All plots with this wavelength only	All plots for the current wavelength.
All plots	All plots.

The window closes, the lines appear across the specified plots, and the sample measurements table is updated with information about the model lines.

Related Topics

Settings for Rate Applications

Data Display

Performing a Scanning Rate Measurement

Performing a Scanning Rate Measurement

Rate measurements can be made on several samples simultaneously using a sample changer or run in a sequential, manual mode.

❖ To perform a scanning Rate measurement

1. Make sure Scan data acquisition is selected on the Type tab in Settings in Rate.
2. Click **Measure Rate**.

3. Load the sample to use to measure the zero.
4. Click **Zero**.

5. Click **Measure**.

If necessary, the Zero action button can be used to remeasure the zero sample.

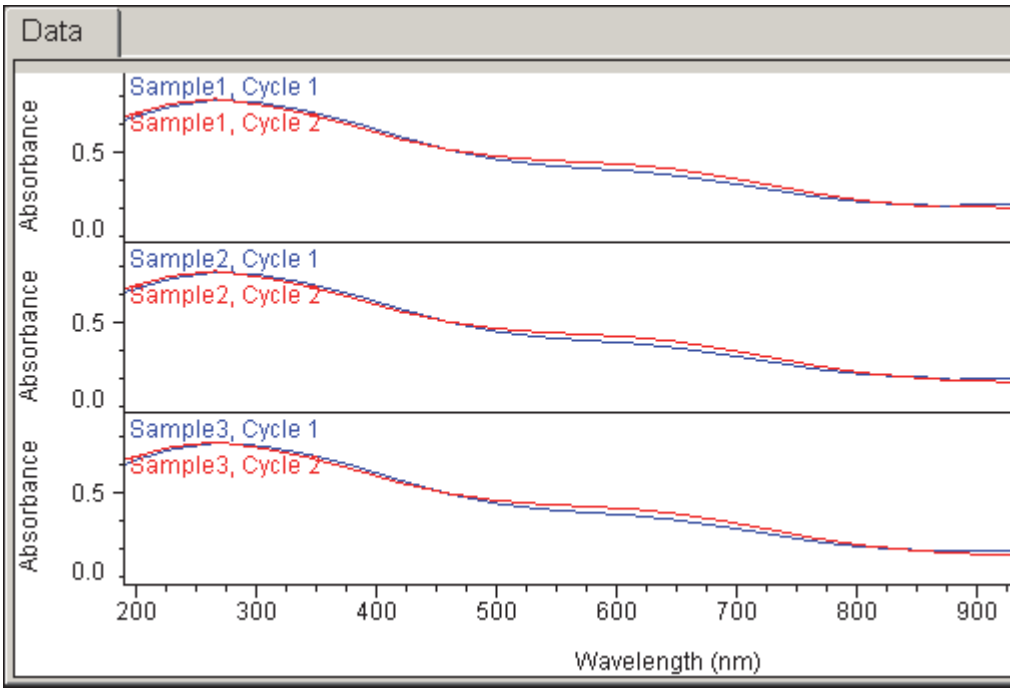
6. When samples information appears, modify it if desired.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

Enter values in any special columns that were specified on the Samples tab in Settings. These values may appear in the rate data report.

7. Click **Continue**.
8. Follow the instructions that appear.

   Each grid on the Data tab displays all the spectra acquired for a particular sample.



   A scroll bar is provided if spectra are out of view.

   Right-click the **Data tab** to access features for changing the scale or adding annotation. See Data Display for more information.

   A sample measurements table appears below the spectra.

Related Topics

Settings for Rate Applications

Data Display

Performing a Fixed Rate Measurement

Adding Custom Calculations

Calculations can be applied to the results from individual samples or standards, results from a group of samples, or between two or more sample results. Depending on your needs, use this table as a guide to the appropriate feature for adding custom calculations.

This feature...	Located here...	Allows you to...
Use sample correction factor	Samples tab	**Specify a multiplication factor for each sample result**. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. The correction factors and corrected results are displayed in the sample measurements table. If a report is generated that includes the samples table, the report will also include the correction factors and corrected results.
Use correction factor	Standards tab	**Specify a multiplication factor for each standard**. The corrected concentration values are used to create the calibration curve. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. Available for Standard Curve, Standard Curve With Two Wavelengths, and Advanced Standard Curve Quant types only. The correction factors and corrected concentration values are displayed in the standards table. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.

This feature...	Located here...	Allows you to...
Calculate additional results	Measurement tab	**Apply pre- or user-defined formulas to all sample measurements** taken with the workbook or template. Available for Fixed, Scan and Quant applications only. For descriptions of the operations and functions usable in user-defined equations, see Operations and Functions. After the measurement, the calculated values and their labels are displayed in the sample measurements table below the data display. If a report is generated that includes the samples table, the report will also include the calculated values and labels.
Advanced Calculations	Navigation pane during a measurement	**Apply predefined formulas to any measurement result or results for any sample or to other calculation results**. Predefined formulas include basic math and statistics. Available for all applications except Rate. The calculated values and any labels that are entered are displayed in the calculations table which appears below the sample measurements table in the right pane. If a report is generated that includes the calculations table, the report will also include the calculated values and labels.

Related Topics

Use sample correction factor

Use (standards) correction factor

Calculate Additional Results

Advanced Calculations

Operations and Functions

The tables below describe the operations and functions usable in equations specified on the Measurement tab for Fixed, Scan and Quant. Arithmetic operations (including functions) are performed first, followed by comparison operations, and finally logical operations.

Note The operators, arguments and functions are not case-sensitive.

Arithmetic Operations

Operator	Description	Example	Returned by Example Operation
+	Addition	2 + 6 + 4	12
-	Subtraction	25 - 4	21
*	Multiplication	25 * 4	100
/	Division	25 / 4	6.25
-	Negation	-4 + 25	21
mod	Modulo division (returns remainder)	16 mod 5	1
&	Text addition	“abc” & “def”	“abcdef”

Comparison Operations

Operator	Description	Example	Result
>=	Greater than or equal	(3 + 2) >= 4	true
<=	Less than or equal	(3 + 2) <= 5	true
=, ==	Equals	(2 + 2) = 4	true
!=, <>	Does not equal	(2 + 2) != 5	true
<	Less than	5 < 4	false
>	Greater than	5 > 4	true
true	The value “true”	(5 > 4) = true	true
false	The value “false”	(5 > 4) = false	false

Logical Operations

Operator	Description	Example	Result
AND	If both sides of the AND are true, result is true	5 > 4 AND 2 = 3	false
OR	If either side of the OR is true, result is true	5 > 4 OR 2 = 3	true

The following equation functions produce a value based on one or more arguments you supply.

Example Function Arguments

Argument	Description
<number>, <angle>, <length>, <value>, <power>, <start>, <len>	An expression that yields a number.
<list>	Any expression that yields a list of numbers.
<text>, <sub>	An expression that yields a text.

Arithmetic Functions

Function	Returned or Performed by Function
abs(<number>)	Absolute value of a number.
ceiling(<number>)	Smallest integer greater than or equal to <number>. Example: Ceiling(2.2) is 3.0
floor(<number>)	Largest integer less than or equal to <number>. Example: Floor(2.7) is 2.0
random()	Random fractional number between 0 and 1.
round(<number>)	Rounds a double value to the nearest integer value.
sqrt(<number>)	Square root of a number.
truncate(<number>)	Truncated number. Everything to the right of the decimal point is removed, leaving only the integer portion of the number. The returned value is not rounded.

Logarithmic Functions

Function	Returned by Function
exp(<number>)	Result of the constant e (2.7182818) raised to the power of the specified number (e^<number>).
ln(<number>)	Natural logarithm (base e logarithm) of a number.
log10(<number>) log(<number>)	Base 10 logarithm of a specified number.
pow(<value>, <power>)	Value raised to the power. Example: pow(2,3) is 8.0

Trigonometric Functions

Note For functions acos and asin, <number> is an expression that yields a number in the range -1 to 1.

Note The formula for converting degrees to radians is radians = degrees * (pi/180).

Function	Returned by Function
acos(<number>)	Arccosine of a number in radians.
asin(<number>)	Arcsine of a number in radians.
atan(<number>)	Arctangent of a number in radians.
atan2(<number1>,<number2>)	Arctangent of <number1> divided by <number2> in radians. The signs of both arguments are used to determine the quadrant of the returned value.
cos(<angle>)	Cosine of an angle measured in radians.
cosh(<angle>)	Hyperbolic cosine of an angle measured in radians.
pi()	Ratio of the circumference of a circle to its diameter.
sin(<angle>)	Sine of an angle measured in radians.
sinh(<angle>)	Hyperbolic sine of an angle measured in radians.
tan(<angle>)	Tangent of an angle measured in radians.
tanh(<angle>)	Hyperbolic tangent of an angle measured in radians.

Statistical Functions

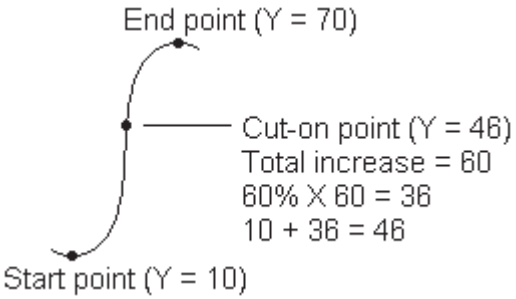
Function	Returned by Function
average(<list>)	Average of a list of numbers (sum divided by the number of items).
max(<list>)	Highest value from two or more numbers.
min(<list>)	Lowest value from two or more numbers.
sum(<list>)	Sum of a list of numbers.
Std. Dev.	Square root of variance, which is determined by taking the average of the squared differences of the values from their average value.
% RSD	Absolute value of the coefficient of variation expressed as a percentage (100*((standard deviation of array X)/(average of array X))).

Text Functions

Function	Returned by Function
FindTextIndex(<search>, <text>)	Index of the first occurrence of the <search> text in the specified text. If the text is not found, a -1 is returned.
Lowercase(<text>)	Copy of the text converted to lower case using the casing rules of the current culture.
PassFail(<expression>)	“Pass” in the language of the current culture if the expression evaluates to true; otherwise, “Fail”.
Quote	Text containing the quote (“) character.
Return	Text containing a carriage return.
TextLength(<text>)	Number of characters, including spaces, in the specified text. A CRLF is counted as two characters.
ExtractText(<text>, <start>, <len>)	Portion of the specified text. The extracted text starts at the specified <start> index and has a specified length.
Tab	Text containing the tab character.
ToNumber(<text>)	Numeric representation of the text.
ToText(<number>)	Text representation of the number.

Measurement Functions

Function	Returned or Performed by Function
Y(location)	Y-axis value at the given location.
Y(location, baseline)	Y-axis value at the given location with a one-point baseline.
Y(location, baseline1, baseline2)	Y-axis value at the given location with a two-point baseline.
Area(start, end)	Area between two spectral limits.
Area(start, end, baseline)	Area between two spectral limits using a one-point baseline.
Area(start, end, baseline1, baseline2)	Area between two spectral limits using a two-point baseline.
PMin(start, end)	Minimum Y-axis value between two spectral limits.
PMax(start, end)	Maximum Y-axis value between two spectral limits.
PMax(start, end, baseline)	Corrects the spectrum with a one-point baseline and then report the maximum Y-axis value between two spectral limits.
PMax(start, end, baseline1, baseline2)	Corrects the spectrum with a two-point baseline and then report the maximum Y-axis value between two spectral limits.
PLoc(start, end)	Location (X-axis value) at which the maximum Y-axis value occurs between two spectral limits.

Function	Returned or Performed by Function
PLoc(start, end, baseline)	Corrects the spectrum with a one-point baseline and then report the location (X-axis value) at which the maximum Y-axis value occurs between two spectral limits.
PLoc(start, end, baseline1, baseline2)	Corrects the spectrum with a two-point baseline and then report the location (X-axis value) at which the maximum Y-axis value occurs between two spectral limits.
PWidth(start, end, percent)	Determines the peak width between two spectral limits. Report the width at which the peak reaches a specified percentage of the peak maximum.
Pavg(start, end)	Determines the average value of the spectrum between two spectral limits.
Pcut(start, end, percent)	Determines the location (X-axis value) of the cut-on or cut-off point in the specified spectral region. For example, for a region with increasing Y value, a cut-on point occurs where the increase reaches the specified percentage of total increase across the region. Here is an example showing a cut-on point with "percent" set to 60: 

Related Topics

Measurement Tab for Fixed

Measurement Tab for Scan

Measurement Tab for Quant

Formulas and Units Tab

Using the Palette Tools and View Finder

Contents

Using the Selection Tool

Use the selection tool (if present) to zoom in or move the spectrum.

Note To display the palette, click  .

To zoom in, draw a box and click inside it. To move the spectrum, drag it up or down.

Related Topics

Using the Spectral Cursor Tool

Using the Peak/Valley Measurement Tool

Using the Peak Area Tool

Using the Region-Threshold Tool

Using the View Finder

Using the Spectral Cursor Tool

Use the spectral cursor tool (if present) to view the X and Y values of a point in a spectrum.

Note To display the palette, click  .

❖ **To view the X and Y values of a point**

1. Select the spectral cursor tool.
2. Click the data display.

Cross hairs appear. The X and Y coordinates of their intersection with the spectrum appear below the palette.

To move the cross hairs, drag across the pane or use the left and right arrow keys on the keyboard.

To remove the cross hairs from the display, select another tool such as the selection tool.

Related Topics

Using the Selection Tool

Using the Peak/Valley Measurement Tool

Using the Peak Area Tool

Using the Region-Threshold Tool

Using the View Finder

Using the Peak/Valley Measurement Tool

Use the peak/valley measurement tool (if present) to measure the height of a peak or depth of a valley from a specified baseline.

Note To display the palette, click  .

❖ To measure a peak or valley

1. Select the peak/valley measurement tool.
2. Click the data display.

   A vertical line appears. The X and Y coordinates of its intersection with the spectrum appear below the palette.
3. Drag the line by its diamond-shaped handle to the peak or valley to measure.
4. Drag the triangular baseline handles to the desired baseline endpoints.

   The height or depth of the peak or valley appears below the palette.

   To annotate the spectrum with the measured value, right-click the spectrum, point to **Annotate** and choose **Tool Value**. See Working With Labels.

   To remove the vertical line and baseline from the display, select another tool such as the selection tool.

Related Topics

Using the Selection Tool

Using the Spectral Cursor Tool

Using the Peak Area Tool

Using the Region-Threshold Tool

Using the View Finder

Using the Peak Area Tool

Use the peak area tool (if present) to measure the corrected area of a peak. This area is bordered by the spectrum, two vertical lines and a baseline.

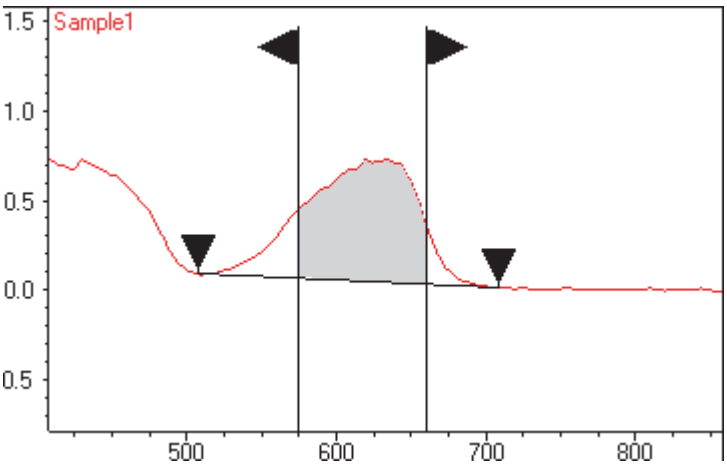
Note To display the palette, click  .

❖ To measure a peak

1. Select the peak area tool.
2. Click the data display.

   Two vertical lines appear (see the illustration below). Their X values define the limits of the region to measure and appear in the Region readout.
3. Drag the lines by their triangular handles to the desired locations.
4. Drag the triangular baseline handles (see the illustration below) to the desired baseline endpoints.

The X values of the endpoints appear in the Baseline readout. The defined area is shaded, and its measurement appears in the Corrected area readout. Here is an example showing the region limits and baseline endpoints adjusted to measure the corrected area of a peak:



To annotate the spectrum with the measured area, right-click the spectrum, point to **Annotate** and choose **Tool Value**. See Working With Labels.

To remove the vertical lines and baseline from the display, select another tool such as the selection tool.

Related Topics

Using the Selection Tool

Using the Spectral Cursor Tool

Using the Peak/Valley Measurement Tool

Using the Region-Threshold Tool

Using the View Finder

Using the Region-Threshold Tool

For data analysis operations such as locating peaks, use the region-threshold tool (if present) to limit the wavelength region.

Note To display the palette, click  .

Drag the vertical lines in the data display or drag horizontally between them to move both at once.

Related Topics

Using the Selection Tool

Using the Spectral Cursor Tool

Using the Peak/Valley Measurement Tool

Using the Peak Area Tool

Using the View Finder

Using the View Finder

For data analysis operations such as locating peaks, use the view finder (if present) to adjust the data display.

Note To display the view finder, click  .

The currently displayed region is indicated by the region markers, the bold vertical lines.

❖ **To expand horizontally**

Click the top half of the **Horizontal Expand/Contract** button. To contract horizontally, click the bottom half.

❖ **To expand vertically**

Click the left half of the **Vertical Expand/Contract** button. To contract vertically, click the right half.

❖ **To expand or contract vertically while keeping the top or bottom of spectra in place**

To expand vertically while keeping the bottom of the spectra in place, click the symbol at the top of this button.

To expand vertically while keeping the top of the spectra in place, click the symbol at the right.

To contract vertically while keeping the bottom of the spectra in place, click the symbol at the bottom.

To contract vertically while keeping the top of the spectra in place, click the symbol at the left.

❖ To display a different region of the same size

There are three ways to do this:

- To move the spectrum to the right, click the top half of the **Roll** button.

  To roll to the left, click the bottom half.

- Drag between the region markers.
- Click to the left of the left region marker or to the right of the right region marker.

❖ To change the display limits by moving the region markers

Drag a region marker left or right.

❖ To display the entire spectrum

Double-click between the region markers.

Related Topics

Using the Selection Tool

Using the Spectral Cursor Tool

Using the Peak/Valley Measurement Tool

Using the Peak Area Tool

Using the Region-Threshold Tool

Setting Application Parameters

To set parameters that determine how data will be acquired or quantified with the current application, click **Settings**.

The tabs in the right pane (and their features) depend on the application.

If  appears on a tab, point to it and read the information to correct the problem.

Action buttons for the current application may include:

Button	Description
	Displays data acquisition features for the application and starts an acquisition.
	Resets the parameters on all the tabs to their default values. For Quant and Rate, also steps through each tab in sequence.
	Makes the parameters on all the tabs available, closes the current workbook, and creates a new workbook.

Settings for Fixed Applications

	To set data acquisition parameters for a Fixed workbook, click **Settings**.

The follow tabs of settings are available:
Measurement Tab for Fixed
Instrument Tab for Fixed
Accessories Tab for Fixed
Samples Tab for Fixed

Change Fixed workbook settings as desired before making sample measurements. All Fixed measurements are saved in a workbook that includes the data acquisition settings.

Measurement Tab for Fixed

	Click **Settings** in Fixed to display the Measurement tab in the right pane.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings (entered when the template was saved using Save Workbook Settings as Template in the File menu).

Feature	Description
Description (optional)	Optional description of the template.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. (See "Operations and Functions" for definitions.) All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. Select the desired formulas. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **OK**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Related Topics

Setting Application Parameters

Settings for Fixed Applications

Operations and Functions

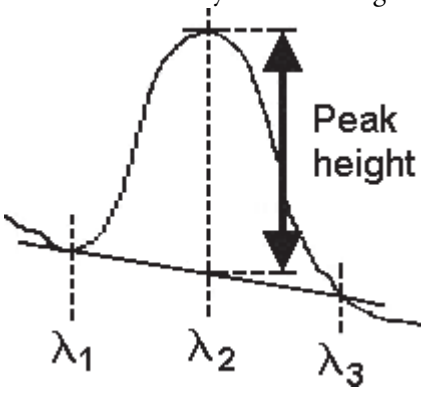
Formulas and Units Tab

Instrument Tab for Fixed

Click **Settings** in Fixed to display the Instrument tab in the right pane.

These settings are available:

Feature	Description
Data mode	Y-axis format for acquired data. Available options include Absorbance and % Transmittance.
Integration time	How long the system acquires and averages data at each measured wavelength. Increasing the integration time improves the signal-to-noise ratio.
Bandwidth	Available options include: • **Bandwidth**. A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features. • **Materials**, **Fiber** and **Micro**. These AFBG (Application Focused Beam Geometry) options match the optical configuration to the application for optimized use of associated accessories. • **Blocked**. Blocks the beam completely.

Feature	Description
Result mode	Determines how results are calculated and displayed: **Normal** displays the results in the units specified in the Data mode setting (see above). **Peak Height** calculates the peak height with respect to a sloping baseline defined by two wavelengths adjacent to the center peak:  The lowest of the three measured wavelengths defines the start of the sloping baseline, the middle wavelength defines the peak location, and the highest wavelength defines the end point of the sloping baseline. **Wavelength 1 + Wavelength 2** adds the Y values at the two measured wavelengths. **Wavelength 1 - Wavelength 2** subtracts the Y value at the second measured wavelength from the Y value at the first. **Wavelength 1 * Wavelength 2** multiplies the Y value at the second measured wavelength times the Y value at the first. **Wavelength 1 / Wavelength 2** divides the Y value at the first measured wavelength by the Y value at the second.
Reference wavelength correction	If available and selected, subtracts the background intensity at the specified reference wavelength from the sample intensity at all measured wavelengths. The reference wavelength value appears in red in the Wavelength Summary table.

Feature	Description
Use control limits	If available and selected, specifies control limits for each wavelength in the table. Enter the desired upper and lower limits for each wavelength in the table, using the appropriate unit for the selected data mode. When the measurement is completed, the limits are listed in the table to the right of the display. Limit lines can also be added to the data display by right-click in the display area, choosing **More Display Options**, and selecting **Show Limit Lines**. The lines indicate whether each measured data point is within specifications.
Wavelength Summary	Wavelengths at which measurements will be made, plus the corresponding control limits if specified (see above). The number of required wavelengths depends on the setting of Result mode (see above). To enter or change the value in a table cell, click it and type. To order the list by wavelength, lower control limit or upper control limit, click the appropriate column heading.

Related Topics

Setting Application Parameters

Settings for Fixed Applications

Accessories Tab for Fixed

Click **Settings** in Fixed to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements.

Related Topics

Setting Application Parameters

Settings for Fixed Applications

Instrument Status Monitors

Samples Tab for Fixed

Click **Settings** in Fixed to display the Samples tab in the right pane.

These settings are available:

Feature	Description
Number of samples	Number of samples for the experiment.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three different samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table on the Samples tab.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Setting Application Parameters

Settings for Fixed Applications

Settings for Scan Applications

To set data acquisition parameters for a Scan workbook, click **Settings**.

The follow tabs of settings are available:
Measurement Tab for Scan
Instrument Tab for Scan
Accessories Tab for Scan
Samples Tab for Scan
Peak Pick Tab for Scan

Measurement Tab for Scan

Click **Settings** in Scan to display the Measurement tab in the right pane.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings (entered when the template was saved using Save Workbook Settings as Template in the File menu).
Description (optional)	Optional description of the template.

Feature	Description
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. (See "Operations and Functions" for definitions.) All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. Select the desired formulas. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **OK**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Feature	Description
Baseline Correction Type	For defining a baseline correction for the raw data. These options are available: • **100%T baseline**. Standard baseline correction. The data may be acquired using an empty holder or using solvent. • **0%T or 0%R baseline**. Corrects for any inherent variations in the electronic zero line of the instrument. More: This can be more of a factor at lower light energy levels present in a DRA (Diffuse Reflectance Accessory) and when working at high absorbance levels. For more information, see ASTM method E903. The calculation in transmission performed in the instrument is: $\frac{S - 0\%T}{100\%T - 0\%T}$ or $\frac{S - 0\%R}{100\%R - 0\%R}$ Requires a previously acquired 100%T baseline. • **Standard reference correction with 0%R or 0%T baseline**. Applies a 100%T baseline correction and a zero line correction (0%R baseline) to a sample measured with a reflectance accessory that is not absolute (for example, a Diffuse Reflectance Accessory). Displays a table for entering a correction value for each measured wavelength. More: The software multiplies the scan result at each data point by the corresponding value in the table (explained below). To delete the information from the table, click **Clear Table**. The calculation in reflectance performed in the instrument is: $\frac{S - 0\%R}{100\%T - 0\%R} \times \text{Std. Ref.}$ Requires a previously acquired 100%T baseline.
Correct single beam substitution error	Allows the ratioed spectrum acquired with the single-beam sphere to be corrected as if it were acquired with a double-beam instrument (where the baseline is collected in the integrating sphere). Requires that Data mode on the Instrument tab be set to Absorbance, % Transmittance, % Reflectance or Log(1/R).

Select **Standard reference correction with 0%R or 0%T baseline** to display additional features:

Feature	Description
Clear Table	Deletes all entries from the table.
Load Table	Fills the table with wavelength and intensity values from a .tsv (tab separated values) file or .csv (comma separated values) file containing X delimiter Y pairs.
Save Table	Saves in a .tsv file, .csv file, or .xml (extensible markup language) file the wavelength and intensity values in the table.

Related Topics

Setting Application Parameters

Settings for Scan Applications

Operations and Functions

Formulas and Units Tab

Instrument Tab for Scan

Click **Settings** in Scan to display the Instrument tab in the right pane.

These settings are available:

Feature	Description
Data mode	Y-axis format for acquired data. Available options include Absorbance and % Transmittance. For Abs*Factor, set **Factor** to a value by which to multiply measured absorbance values.
Smooth	Select the desired degree of smoothing (if available for the selected data mode and Derivative setting). Smoothing reduces noise in the data but can also remove some spectral features such as small peaks, valleys and shoulders. For more information, see About Smoothing.
Derivative	Select the desired derivative order for acquired data (if available for the selected data mode).
Start wavelength and End wavelength	Starting and ending values of the wavelength range for acquiring data.

Feature	Description
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features, especially in scanning measurements.
Integration time	How long the system acquires and averages data at each data interval. Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval	Difference in wavelength between two consecutive data points.
Scan speed	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.

Related Topics

Setting Application Parameters

Settings for Scan Applications

Accessories Tab for Scan

Click **Settings** in Scan to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements.

Related Topics

Setting Application Parameters

Settings for Scan Applications

Instrument Status Monitors

Samples Tab for Scan

Click **Settings** in Scan to display the Samples tab in the right pane.

These settings are available:

Feature	Description
Number of samples	Number of samples for the experiment.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table on the Samples tab.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Setting Application Parameters

Settings for Scan Applications

Peak Pick Tab for Scan

When setting up a Scan workbook, click **Settings** to display the Peak Pick tab in the right pane.

These settings are available:

Feature	Description
Result	Data analysis operation to perform automatically after sample measurement. Select **Off** to not perform any operation. The parameters for a selected operation appear to the right. See Finding Peaks in Scan Data or Finding Value Level Crossings in Scan Data for information on setting them and how results are displayed.

Related Topics

Setting Application Parameters

Settings for Scan Applications

Settings for Quant Applications

To set parameters that define a Quant template, click **Settings**.

The follow tabs of settings are available:
Type Tab for Quant
Measurement Tab for Quant
Instrument Tab for Quant
Accessories Tab for Quant
Standards Tab for Quant
Samples Tab for Quant

Note Change Quant settings only before sample measurements. All Quant measurements saved in a workbook must be made with the same settings. See Setting Application Parameters for information about unlocking settings.

Related Topics

Formulas and Units Tab

Performing a Quantitative Analysis

Type Tab for Quant

Click **Settings** in Quant to display the Type tab in the right pane.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings (entered when the template was saved using Save Workbook Settings as Template in the File menu).
Description (optional)	Optional description of the template.
Quant Type	Type of quantitative analysis (see the table below). An image representing the selected type appears to the right. The required tabs also appear (click the **Reset** action button to step through them).
Pathlength	Distance the light travels through the sample.

Quant Type	How the Type Calculates Sample Concentration
Manually entered factor	Uses the equation C = K * A, where C is the concentration of the analyte in the selected units, K is the entered factor, and A is the absorbance.
Measure single standard	Divides the sample absorbance value by the average absorbance value of a single standard. This is in effect a "Standard curve" quantification (see below) with just one standard.
Standard curve	Uses a simple standard curve based on the absorbance of a set of standards at a specified wavelength.
Standard curve with two wavelengths	Takes the average of values determined by each of two standard curves associated with the specified wavelengths.
Advanced standard curve	Uses a standard curve generated from a specified equation.

Related Topics

Settings for Quant Applications

Formulas and Units Tab

Performing a Quantitative Analysis

Measurement Tab for Quant

Click **Settings** in Quant to display the Measurement tab in the right pane. The available features depend on the Quant Type setting on the Type tab. The features for each type are described in tables below.

These settings are available:

Manually entered factor

Feature	Description
Analysis wavelength(s)	Wavelength(s) to use for the quantitative analysis.
Correction	Baseline correction to apply to raw data. Available options: • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None** Uses uncorrected data.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Use user-defined factor	Calculates component concentration using the entered factor (K in the equation C = K *A), with the selected unit.
Use extinction coefficient	Calculates component concentration with absorbance (A) equal to $\varepsilon c \ell$ where ε is the entered extinction coefficient (a constant dependent on the component and wavelength), c is concentration in the selected unit, and ℓ is 1 cm (the pathlength).

Feature	Description
Molecular weight	Molecular weight of the component. Available only if the units specified on this tab are different and require this value for a conversion.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. (See "Operations and Functions" for definitions.) All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. Select the desired formulas. Hold down the Ctrl or Shift keys on the keyboard while making multiple selections. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **OK**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available formulas depend on the Formulas & Units tab settings.

Measure single standard

Feature	Description
Analysis wavelength(s)	Wavelength(s) to use for the quantitative analysis.
Correction	See Correction above.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Standard concentration	Concentration of the single standard.
Std averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.
Calculate additional results	See Calculate additional results above.

Standard curve

Feature	Description
Analysis wavelength(s)	Wavelength(s) to use for the quantitative analysis.
Correction	See Correction above.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	See Calculate additional results above.

Standard curve with two wavelengths

Feature	Description
Analysis wavelengths	Wavelengths to use for the quantitative analysis.
Correction	See Correction above.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings.
Calculate additional results	See Calculate additional results above.

Advanced standard curve

Feature	Description
Standard Curve Equation	Equation used to quantify samples, written in a form similar to that used in a command script language, with constants, mathematical functions, etc. (See Operations and Functions for definitions of operations and functions.) All functions are not case-sensitive. Spaces are not allowed between a function name and "(". For this Quant type the equation defines the value for the Y-axis of the calibration curve. Instructions: ❖ **To use a predefined equation** 1. Click **Select**. 2. Select the desired equation and click **OK**. The available equations depend on the Formulas & Units tab settings in Options. ❖ **To define an equation** 1. Click **Build**. 2. To enter an equation in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided equation, select it and enter values for the variables. The available equations depend on the Formulas & Units tab settings. 3. Click **OK**.
Component name	Component to quantify.
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	See Calculate additional results above.

Related Topics

Operations and Functions

Formulas and Units Tab

Settings for Quant Applications

Performing a Quantitative Analysis

Instrument Tab for Quant

Click **Settings** in Quant to display the Instrument tab in the right pane.

These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data. Available options include Absorbance and % Transmittance. For Abs*Factor (Scan only), set **Factor** to a value by which to multiply measured absorbance values.
Smooth (Scan only)	Select the desired degree of smoothing (if available for the selected data mode and Derivative setting). Smoothing reduces noise in the data but can also remove some spectral features such as small peaks, valleys and shoulders. See About Smoothing.
Derivative (Scan only)	Select the desired derivative order for acquired data (if available for the selected data mode).
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for Scan) or at each measured wavelength (for Fixed). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Wavelength Summary (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Settings for Quant Applications

Formulas and Units Tab

Performing a Quantitative Analysis

Accessories Tab for Quant

Click **Settings** in Quant to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements.

Related Topics

Settings for Quant Applications

Instrument Status Monitors

Formulas and Units Tab

Performing a Quantitative Analysis

Standards Tab for Quant

To display the Standards tab in the right pane, click **Settings** in Quant and then set **Quant Type** to Standard curve, Standard curve with two wavelengths, or Advanced standard curve.

These settings are available:

Feature	Description
Curve fit type	Type of equation used to create the standard curve from standard concentration values.
Standard averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.

Feature	Description
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.
Use correction factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Contains concentration and other information about the standards. To add information from a .csv (comma separated values) file or .tsv (tab separated values) file, click **Import Standards**. To save the information in a .csv file or .tsv file, click **Export Standards**.

Related Topics

Settings for Quant Applications

Formulas and Units Tab

Performing a Quantitative Analysis

Samples Tab for Quant

Click **Settings** in Quant to display the Samples tab in the right pane.

These settings are available:

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use weight/volume correction	If available for the current unit, corrects sample concentrations using the entered target weight and volume: corrected concentration = measured concentration * (actual weight / target weight) * (target volume / actual volume)
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Settings for Quant Applications

Formulas and Units Tab

Performing a Quantitative Analysis

Settings for Rate Applications

To set parameters for the current Rate workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Rate
Measurement Tab for Rate
Instrument Tab for Rate
Accessories Tab for Rate
Samples Tab for Rate
Rate Calculations Tab for Rate

Related Topics

Rate

Performing a Rate Measurement

Type Tab for Rate

Click **Settings** in Rate to display the Type tab in the right pane.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings (entered when the template was saved using Save Workbook Settings as Template in the File menu).
Description	Description of the template.
Rate Type	Type of rate measurement to perform (see the table below). An image representing the selected type appears to the right. The required tabs also appear (click the Reset action button to step through them).
Mode	These options are available: **Time**. Performs rate measurements based on elapsed time. **Temperature**. Performs rate measurements based on elapsed time and also records temperature settings. Available for fixed rate experiments only.

Rate Type	How the Type Collects Data
Single wavelength	For each sample, measures a specified wavelength at specified intervals of time.
Multiple wavelengths	For each sample, measures multiple specified wavelengths at specified intervals of time.
Scan data acquisition	For each sample, measures a range of wavelengths at specified intervals of time.

Related Topics

Settings for Rate Applications

Performing a Rate Measurement

Modifying a Rate Curve

Measurement Tab for Rate

Click **Settings** in Rate to display the Measurement tab in the right pane.

These settings are available:

Feature	Description
Integration time	How long the system acquires and averages data to obtain each data point at each measured wavelength. Available for single and multicell experiments only. (To set integration time for scan rate experiments, see Instrument Tab for Rate.)
Dwell time	How long the sample remains in the light beam. Available for single and multicell experiments only. Use this setting to vary the number of data points collected per cell per cycle. Dwell time is always a multiple of integration time (see above). Dwell time can be very useful when performing multicell rate measurements. This is because the cell changer accessory will position a cell in the light beam for a specified period of time (dwell time), rather than moving back and forth between cells and increasing the time between measurements (when no sample is in the light beam). For example, if the integration time is 0.1 second and dwell time is 0.5 second, five data points will be recorded for each cell before the cell changer moves to the next cell. For fairly fast reactions, multiple samples can be measured, with over ten times as many data points acquired within a short time. This can improve the accuracy of rate calculations for faster multicell rate measurements.
Number of stages	Defines the number of measurement stages over which the frequency of data acquisition can be determined. For example, if a reaction has two components, the first component could be fast and require a high data density. The second component could be much slower and longer lived, therefore requiring a lower data density. Defining multiple stages enables the use of the proper data density over a given period of time. Available for single and multiple wavelength experiments only.
Temp unit	Determines the temperature unit used in the stages table (see below).
Time unit	Determines the time unit used in the stages table (see below).

Feature	Description
Stages table	Available for single and multiple wavelength experiments only. For time-based experiments: Defines for each stage the start time, end time and interval (how frequently a measurement is made). For example, using an interval of 10 seconds would measure the sample every 10 seconds from the start time to the end time. In a multicell experiment the interval setting is the measurement interval for each sample. For temperature-based experiments: Defines for each stage the end temperature, ramp rate, hold type, hold time, interval and whether data collection will occur. **Target Temp**. Enter a target temperature between 0.00 °C (32.00 °F or 273.15 °K) and 110.00 °C (230.00 °F or 383.15 °K). **Ramp Rate**. Enter a ramp rate from 0.40 to 20.00 °C/min (0.72 to 36.00 °F/min or 0.40 to 20.00 °K/min) up or down. **Hold Type**. Specify a hold time at the target temperature before continuing to the next stage. **Time** holds for a specified time (see Hold Time below). **Prompt** holds until the operator responds to a message in the software. **Trigger** waits for an external trigger. Choosing Start in the trigger prompt will override the trigger. **Hold Time**. When Hold Type is set to Time, enter the length of time to hold at the target temperature before continuing to the next stage. **Interval**. Specifies how frequently a measurement is made. Must be equal to or greater than the specified Integration Time and less than the stage duration. Disabled when **Collect Data** (see below) is set to No. **Collect Data**. Specifies whether to collect data during each stage.
Cycle time (Scan only)	Time between each measurement cycle. Available for scan experiments only.
Number of cycles (Scan only)	Number of measurement cycles. Available for scan experiments only.
Duration (Scan only)	Length of the experiment, calculated by multiplying the cycle time by the number of cycles (see above). Available for scan experiments only.

Related Topics

Settings for Rate Applications

Performing a Rate Measurement

Modifying a Rate Curve

Instrument Tab for Rate

Click **Settings** in Rate to display the Instrument tab in the right pane.

These settings are available:

Feature	Description
Data mode	Y-axis format for acquired data. Available options include Absorbance and % Transmittance. For Abs*Factor (Scan only), set **Factor** to a value by which to multiply measured absorbance values.
Smooth (Scan only)	Select the desired degree of smoothing. Smoothing reduces noise in the data but can also remove some spectral features such as small peaks, valleys and shoulders. For more information, see About Smoothing. Available for scan experiments only.
Derivative (Scan only)	Select the desired derivative order for acquired data. Available for scan experiments only.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data. Available for scan experiments only.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features, especially in scanning measurements.
Integration time (Scan only)	How long the system acquires and averages data at each data interval. Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed. Available for scan experiments only.
Data interval (Scan only)	Difference in wavelength between two consecutive data points. Available for scan experiments only.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases. Available for scan experiments only.

Feature	Description
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed. Available for scan experiments only.
Reference wavelength correction	When selected, automatically acquires a reference measurement at a specified wavelength each time a sample measurement is taken. The reference measurement is then subtracted from the corresponding sample measurement to produce a corrected measurement result. Only the corrected results are reported. Use the **Reference wavelength** box to specify a wavelength for the reference measurement. Available for single and multiple wavelength experiments only.
Wavelength Summary	Wavelengths to monitor during the reaction. Available for single and multiple wavelength experiments only.

Related Topics

Settings for Rate Applications

Performing a Rate Measurement

Modifying a Rate Curve

Accessories Tab for Rate

Click **Settings** in Rate to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements.

Related Topics

Instrument Status Monitors

Settings for Rate Applications

Performing a Rate Measurement

Modifying a Rate Curve

Samples Tab for Rate

Click **Settings** in Rate to display the Samples tab in the right pane.

These settings are available:

Feature	Description
Number of samples	Number of samples for the experiment.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Set up sample table columns	Specifies the columns of sample information to be entered during the experiment. To add a column, type the desired name in an empty cell and press the Tab key. To delete a column (except the Sample Title column), right-click it and choose **Delete Column**. To delete all the columns (except the Sample Title column), right-click the table and choose **Delete All Columns**.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table on the Samples tab.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. Additional columns are defined by the **Set up sample table columns** option (see above). To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Settings for Rate Applications

Performing a Rate Measurement

Modifying a Rate Curve

Rate Calculations Tab for Rate

Click **Settings** in Rate to display the Rate Calculations tab in the right pane (for time-based single and multiple wavelength experiments only).

These settings are available:

Feature	Description
Calculate rate constants automatically at end of data acquisition	Calculates rate constants automatically at the end of the experiment. When this option is selected, the rate curve appears in the data display and the rate settings and calculations appear in the sample measurements table after the measurement is completed. To modify the rate curve, **double-click the data display** or choose **Analyze (menu) > Modify Rate Curve**. When this option is cleared and rate calculations have been entered, use the **Modify Rate Curve** window to view, modify, update and accept the rate calculations settings. After you accept the settings, the window closes and the rate curve appears in the data display and the rate settings and calculations appear in the sample measurements table. See Performing Rate Measurements for more information.
Calculate Rate	For each measurement stage, enter the starting and ending times of the rate vector, the rate scaling factor, and the order of the reaction. The factor is a constant that is multiplied by a calculated kinetics parameter to give physical significance to the measurement. To enter different settings for individual wavelengths, select **Specify settings for individual wavelengths** (available only if Rate Type on the Type tab is set to Multiple wavelengths). In the Wavelength column, select or type the desired wavelength for each row of settings. The available wavelengths are those entered on the Instrument tab. To delete a row of information from the table, right-click the row and choose **Delete Selected Row**. To delete all the information from the table, right-click it and choose **Clear Table**.

Related Topics

Settings for Rate Applications

Performing a Rate Measurement

Modifying a Rate Curve

Configuring a Report

A report contains a table of sample data and other specified information. Reports can be saved and printed or the sample data can be exported.

Note See the Report Master Page tab in Options to define global settings for all reports including headers and footers and a logo or other image.

❖ To configure a report

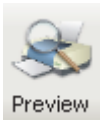
1. Open a workbook or measure a sample.
2. Click **Reports**.

   The sample data that were measured and archived in the opened workbook are displayed on the Samples tab.
3. Select the sample results to include by clicking a row in the table.

   Use “Shift + click” or “Ctrl + Click” to select additional rows.
4. To specify the columns to include and their order, click the Reportable Data tab.
5. To specify the items to include in printed reports for this workbook, click the Layout tab.

   Note To specify that all items in printed reports span the full width of each page, select **Fit To Width** in the navigation pane. See Layout tab for more information about formatting options for printed report.
6. To preview all the pages in the report, click the **Preview** action button.

7. To print the report, click the **Print** action button.

Related Topics

Report Master Page Tab

Reports

Exporting Data

You can export acquired data and associated measurement results from any opened report or configure INSIGHT to export all data and results automatically.

Contents

Exporting Data from a Report

Acquired data and associated measurement results can be manually saved in a specified format and location.

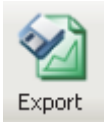
❖ To export acquired data and measurement results

1. Open a workbook or measure a sample.
2. Click **Reports**.

3. Select the sample data to be exported by clicking a row in the table.

   Use **Shift + Click** or **Ctrl + Click** to select additional rows.

4. Click the **Export** action button.



5. Select a location for the exported file.
6. Type a file name for the exported data in the **File name** box.
7. Set **Save as type** to the desired file format (several formats for exporting reports and spectra are available).
8. Click **Save**.

   The information is saved in a file in the specified format, file name and location.

Related Topics

Exporting Data

Reports

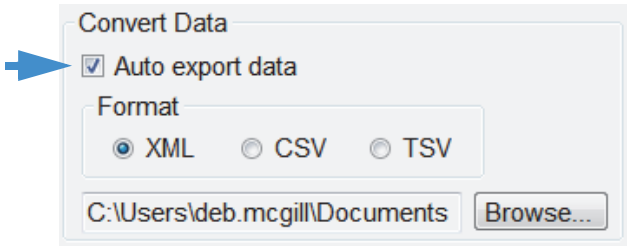
Exporting Data Automatically

INSIGHT software can be configured to automatically save all acquired data in a specified format and location.

❖ **To configure INSIGHT to export all acquired data**

1. From any screen, click **Options**.

2. Click the **Data Store** tab.
3. In the Convert Data group, select **Auto export data**.



4. Select an available format.
5. To select the location for the exported data, type a new path or use **Browse**.

   The results for each measured sample are saved in a separate file in the specified format and location. The filename includes the sample and workbook name and the date and time.

Related Topics

Exporting Data

Reports

Managing Data

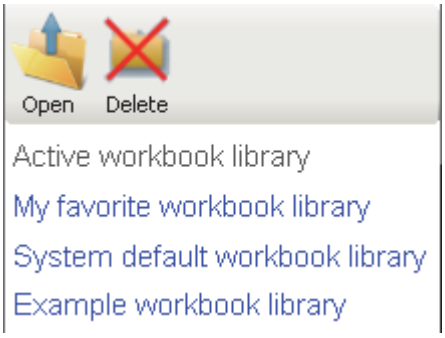
Contents

My Data

Use **My Data** to access saved workbooks, templates and Performance Verification (PV) result reports. Drag the borders of the panes to change their size and shape.

Use these tabs to select items to open or delete:

Tab	Description
Workbooks	Select a folder in the tree to list its workbooks. Select a listed workbook to see its spectra. To zoom in on the displayed spectra, draw a box and click inside it or use the view finder. To view the full data range, double-click the spectral display.
Templates	Select a group folder in the tree to list its templates.
PV Reports	Select the listed result reports of interest. To select multiple reports, hold down **Shift** or **Ctrl**. (One window will display all the opened results.)



Click **Open** to open the selected workbook or template (and associated application) or the selected PV reports. Alternatively, double-click the item to open it. Additional measurements can be appended to an opened workbook. Opened PV results can be printed and copied from the report window.

Click **Delete** to delete the selected workbook or template.

Use the links below the buttons to display archived workbooks. Click **Active workbook library** to view the directory where a workbook was last saved. To change the path to the default workbook, right-click **My favorite workbook library** and choose **Edit Link**.

Related Topics

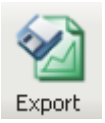
Managing Data

Reports

A report contains a table of sample data and other specified information.

To configure or view the report after opening a workbook or measuring a sample, click **Reports**.

These action buttons appear in Reports:

Button	Description
	Shows the current report before printing, with the header and footer specified on the Report Master Page tab in Options.
	Prints the current report on the specified printer.
	Saves the specified spectral data or sample measurement results in the specified format (see the table below).

The export formats include:

Format	Description
Report, Excel XML Spreadsheet (*.xml)	Can be opened in Excel®. Only the columns selected on the Reportable Data tab are saved and only for the rows selected on the Samples tab. Configure those tabs to include the desired information before exporting. For Rate applications, the data is displayed with one column per spectrum, and one sample per sheet.
Report, Tab Separated Values (*.tsv)	Can be opened in Notepad or Excel. Only the columns selected on the Reportable Data tab are saved and only for the rows selected on the Samples tab. Configure those tabs to include the desired information before exporting.
Report, Comma Separated Values (*.csv)	Can be opened in Notepad or Excel. Only the columns selected on the Reportable Data tab are saved and only for the rows selected on the Samples tab. Configure those tabs to include the desired information before exporting.
Spectrum, Excel XML Spreadsheet (*.xml)	Can be opened in Excel. Saves absorbance values along with the corresponding wavelengths only for the rows selected on the Samples tab. If multiple sample rows are selected, the corresponding absorbance values for each wavelength are saved onto separate worksheets within one Excel file.
Spectrum, Tab Separated Values (*.tsv)	Saves absorbance values along with the corresponding wavelengths for the rows selected on the Samples tab in a format that can be opened in Notepad or Excel. If multiple sample rows are selected, the corresponding absorbance values for each wavelength are saved sequentially in one column.

Format	Description
Spectrum, Comma Separated Values (*.csv)	Saves absorbance values along with the corresponding wavelengths for the rows selected on the Samples tab in a format that can be opened in Notepad or Excel. If multiple sample rows are selected, the corresponding absorbance values for each wavelength are saved sequentially in one column.
Spectra, New Workbook (*iwbk)	Saves the settings and information about any samples, standards and advanced calculations for the selected application to a new INSIGHT workbook.
Spectrum 3D, Excel XML Spreadsheet (*.xml)	Saves absorbance values along with the corresponding time and wavelengths for the rows selected on the samples tab in a format that can be opened in Excel. Only available in Scan Rate applications.

If the computer is not mapped to recognize that .xml files should be opened with Excel, open the exported file from within Excel.

These tabs appear in the Reports right pane:

Tab	Description
Samples	Displays the sample data archived in the opened workbook. To sort the information in the table, click the desired column heading. To view particular data, select it in the table. For most applications this displays a sample spectrum. For fixed Rate data, this displays spectral data points for the indicated wavelength and the line resulting from the rate calculations, if specified. For scan Rate data, all of the sample's spectra appear. See Data Display for information on using features made available by right-clicking the displayed data. To view, print or copy data acquisition and other information about data, right-click it in the list and choose **Properties**. To change a sample ID, click its cell and type a new ID. (If sample IDs are not editable, choose **Options > Preferences** and clear **Prevent removal of data**.)
Reportable Data	Specifies the report columns to include and their order. The available columns depend on the current application and workbook settings. To include a column, select it in the **Available Columns** list and click  . To remove an included column, select it in the **Reported Columns** list and click  . To move an included column up, select it and click  . To move an included column down, select it and click  . To restore the most recent configuration, click  . Only the information associated with selected columns will be included in exported files. See the information on exporting reports above.
Layout	Specifies the items to include in printed reports (see the table below).

The Layout tab includes:

Feature	Description
Header/Footer	Includes a header and footer in the printout.
Header Items	Title and subtitle to appear in the header. Use the **Font** buttons to specify their fonts.

Feature	Description
Settings info	Includes a table of workbook settings in printed reports including the name, serial number and settings for any accessories that were used. Use the **Font** button to specify the font for the information.
Samples table	Includes a table of sample measurement information for the rows selected on the Reports > Samples tab in printed reports. Use the **Font** button to specify the font for the information.
Results/ Calculations table	This option adds the following if available to printed reports: • **Results table**, which appears to the right of the data display below the Sample ID readout. The results table shows the results of analysis operations and whether sample points fall within specified control limits. • **Calculations table**, which appears below the sample measurements table when Advanced Calculations is selected in the navigation pane (available for all applications except Rate and DNA Melting). Use the **Font** button to specify the font for the information.
Standards	For Quant applications that include standards, includes a table and graph of information for the standards in printed reports. Use the **Font** button to specify the font for the information.
X-Y pairs	For Rate applications, includes a table of x-y data for each sample row selected on the Samples tab in printed reports. For each sample, one X-Y pair is logged for each wavelength, sampling interval and stage. Use the **Font** button to specify the font for the information.
Graphs	Includes sample spectra and other data plots in printed reports. Data can be placed in individual graphs or overlaid on one graph. • Select **Overlay** to print data plots overlaid. (Note that this is the only setting that allows tables and graphs to be printed on the same page.) • Select **Separate** to print each data plot in a separate graph, with the specified number of graphs on a page.
Report notes	Includes a Report Notes box in printed reports. To add text to the Report Notes box, click **Edit**. Use the **Font** button to specify the font for the information.
Page Setup	Specifies the paper size, orientation and other printing attributes.
Fit to page width	Changes the layout of all included items so they fill the page width. (Column headings may be truncated.)

Note We recommend first defining a master page on the Report Master Page tab in Options.

Related Topics

Configuring a Report

Managing Data

Math and Analysis Operations

Contents

Smoothing Scan Data

Use **Smooth** in the Math menu to reduce noise in wavelength scan data. This feature is available in Scan and Scan Rate applications.

The smoothing algorithm uses the Savitzky-Golay method. For more information, see About Smoothing.

❖ To smooth data

1. Choose **Math** (menu) **>** **Smooth**.

   The selected spectrum appears in the Operands pane.

The workbook spectra are listed at the left. To add others:

   a. Click **Browse**.

   b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

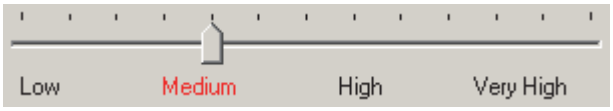
c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

2. Select any other listed spectra to smooth and click **Add**.

   To remove a spectrum from the Operands pane, click it and click **Remove**. To remove all the spectra, click **Clear All**.

3. Select the polynomial order.

4. Specify the degree of smoothing (number of points in the smoothing formula) by dragging the slider:



5. To perform a math operation on the smoothed result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

> **Note** To use the result in another Smooth operation, use **Workbook** or **Temporary** to add it to the list (see step 6), click **Add** to make it an operand, and continue with step 2.

   Perform the operation as explained in the appropriate related topic below.

   To return to the previous window, click **Back**.

   To perform additional math operations, repeat this step.

6. To save the result in the current workbook, click **Workbook** and name the spectrum or spectra when prompted.

   The data is added to the list and is available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

7. Click **Close** to close each displayed math window.

Related Topics

About Smoothing

Math and Analysis Operations

About Smoothing

The smoothing feature of INSIGHT software uses a Savitzky-Golay algorithm modified to reduce high frequency breakthrough; that is, to reduce noise in the spectrum. The algorithm is based on performing a least-squares linear regression fit of a polynomial of order k over at least k+1 data points around each point in the spectrum.

Converting Scan Data to a Derivative

Use **Derivative** in the Math menu to convert scan data to the first, second, third or fourth derivative. This feature is available in Scan and Scan Rate applications.

❖ To convert scan data to a derivative

1. Choose **Math** (menu) > **Derivative.**

   The selected spectrum appears in the Operands pane.

The workbook spectra are listed at the left. To add others:

   a. Click **Browse**.

   b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

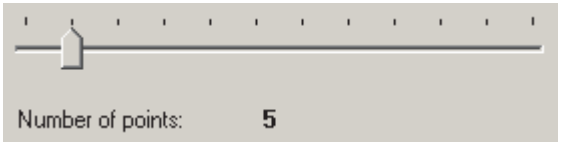
2. Select any other listed spectra to convert and click **Add**.

   To remove a spectrum from the Operands pane, click it and click **Remove**. To remove all the spectra, click **Clear All**.

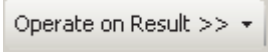
3. Select the derivative order.

4. Select the polynomial order of the derivative formula.

5. Specify the number of points in the derivative formula by dragging the slider:



6. To perform a math operation on the converted result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.



Note To use the result in another Derivative operation, use **Workbook** or **Temporary** to add it to the list (see step 7), click **Add** to make it an operand, and continue with step 2.

Perform the operation as explained in the appropriate related topic below.

To return to the previous window, click **Back**.

To perform additional math operations, repeat this step.

7. To save the result in the current workbook, click **Workbook** and name the spectrum or spectra when prompted.

   The data is added to the list and is available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

8. Click **Close** to close each displayed math window.

Related Topics

Math and Analysis Operations

Converting Scan Data to Other Units

Use **Convert Spectra** in the Math menu to convert scan data to another Y-axis format.

❖ **To convert scan data to another format**

1. Choose **Math** (menu) **> Convert Spectra.**

   The selected spectrum appears in the Operands pane.

The workbook spectra are listed at the left. To add others:

   a. Click **Browse**.

   b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

2. Select any other listed spectra to convert and click **Add**.

   To remove a spectrum from the Operands pane, click it and click **Remove**. To remove all the spectra, click **Clear All**.

3. Select the desired Y-axis format.

4. To perform a math operation on the converted result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

Note To use the result in another Convert Spectra operation, use **Workbook** or **Temporary** to add it to the list (see step 5), click **Add** to make it an operand, and continue with step 2.

Perform the operation as explained in the appropriate related topic below.

To return to the previous window, click **Back**.

To perform additional math operations, repeat this step.

5. To save the result in the current workbook, click **Workbook** and name the spectrum or spectra when prompted.

   The data is added to the list and is available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

6. Click **Close** to close each displayed math window.

Related Topics

Math and Analysis Operations

Adding Two Scan Spectra

Use **Add** in the Math menu to add two scan spectra.

❖ To add two scan spectra

1. Choose **Math** (menu) **> Add.**

   The selected spectrum appears in the Operand 1 pane. To use a different listed spectrum, select it and click **Add** in the Operand 1 box.

The list includes only current workbook spectra. To add others:

   a. Click **Browse**.

   b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

2. Select the second spectrum to add and click **Add** in the Operand 2 box.

3. To multiply the second spectrum by a factor other than 1, drag the slider or use **Factor**.

Type a value in the **Factor** text box or use its up and down arrow buttons. Use **Increment** to set the amount of change these buttons make to the factor when clicked. For example, with Factor set to 1.000 and Increment set to 0.500, clicking the Factor up arrow button changes the factor to 1.500.

4. To perform a math operation on the addition result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

Note To use the result in another Add operation, use **Workbook** or **Temporary** to add it to the list (see step 5), click **Add** in the Operand 1 box, and continue with step 2.

Perform the operation as explained in the appropriate related topic below.

To return to the previous window, click **Back**.

To perform additional math operations, repeat this step.

5. To save the result in the current workbook, click **Workbook** and name the spectrum when prompted.

The data is added to the list and is available for operations.

To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

6. Click **Close** to close each displayed math window.

Related Topics

Math and Analysis Operations

Normalizing Scan Data

Use **Normalize** in the Math menu to adjust the Y scale of a scan spectrum so that a selected data point has the desired Y value.

❖ To normalize scan data

1. Choose **Math** (menu) **> Normalize.**

The selected spectrum appears in the Operands pane.

The workbook spectra are listed at the left. To add others:

a. Click **Browse**.

b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

2. Select any other listed spectra to normalize and click **Add**.

   To remove a spectrum from the Operands pane, click it and click **Remove**. To remove all the spectra, click **Clear All**.

3. Select the desired Y value for the data point specified in the next step.

4. Select the wavelength of the data point.

5. To perform a math operation on the normalized result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

   Note To use the result in another Normalize operation, use **Workbook** or **Temporary** to add it to the list (see step 6), click **Add** to make it an operand, and continue with step 2.

   Perform the operation as explained in the appropriate related topic below.

   To return to the previous window, click **Back**.

   To perform additional math operations, repeat this step.

6. To save the result in the current workbook, click **Workbook** and name the spectrum or spectra when prompted.

   The data is added to the list and is available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

7. Click **Close** to close each displayed math window.

Related Topics

Math and Analysis Operations

Subtracting a Scan Spectrum

Use **Subtract** in the Math menu to subtract a scan spectrum from another.

❖ To subtract a scan spectrum

1. Choose **Math** (menu) **> Subtract.**

   The selected spectrum appears in the Operand 1 pane. To use a different listed spectrum, select it and click **Add** in the Operand 1 box.

The list includes only current workbook spectra. To add others:

a. Click **Browse**.

b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

2. Select the spectrum to subtract and click **Add** in the Operand 2 box.

3. To multiply the second spectrum by a factor other than 1, drag the slider or use **Factor**.

   Type a value in the **Factor** text box or use its up and down arrow buttons. Use **Increment** to set the amount of change these buttons make to the factor when clicked. For example, with Factor set to 1.000 and Increment set to 0.500, clicking the Factor up arrow button changes the factor to 1.500.

4. To perform a math operation on the subtraction result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

   Note To use the result in another Subtract operation, use **Workbook** or **Temporary** to add it to the list (see step 5), click **Add** in the Operand 1 box, and continue with step 2.

   Perform the operation as explained in the appropriate related topic below.

   To return to the previous window, click **Back**.

   To perform additional math operations, repeat this step.

5. To save the result in the current workbook, click **Workbook** and name the spectrum when prompted.

   The data is added to the list and is available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

6. Click **Close** to close each displayed math window.

Related Topics

Math and Analysis Operations

Ratioing Two Scan Spectra

Use **Ratio** in the Math menu to divide a scan spectrum by another.

❖ To ratio two scan spectra

1. Choose **Math** (menu) **> Ratio.**

   The selected spectrum appears in the Operand 1 pane. To use a different listed spectrum, select it and click **Add** in the Operand 1 box.

The list includes only current workbook spectra. To add others:

   a. Click **Browse**.

   b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

2. Select the spectrum by which to divide the first and click **Add** in the Operand 2 box.

3. To multiply the second spectrum by a factor other than 1, drag the slider or use **Factor**.

   Type a value in the **Factor** text box or use its up and down arrow buttons. Use **Increment** to set the amount of change these buttons make to the factor when clicked. For example, with Factor set to 1.000 and Increment set to 0.500, clicking the Factor up arrow button changes the factor to 1.500.

4. To perform a math operation on the division result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

   Note To use the result in another Ratio operation, use **Workbook** or **Temporary** to add it to the list (see step 5), click **Add** in the Operand 1 box, and continue with step 2.

   Perform the operation as explained in the appropriate related topic below.

   To return to the previous window, click **Back**.

   To perform additional math operations, repeat this step.

5. To save the result in the current workbook, click **Workbook** and name the spectrum when prompted.

   The data is added to the list and is available for operations.

   To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

6. Click **Close** to close each displayed math window.

Related Topics

Math and Analysis Operations

Averaging Scan Spectra

Use **Average** in the Math menu to find the average of two or more scan spectra.

❖ To use Average

1. Choose **Math** (menu) **> Average.**

   The selected spectrum appears in the Operands pane.

   The workbook spectra are listed at the left. To add others:

   a. Click **Browse**.

   b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

2. Select any other listed spectra to average and click **Add**.

   To remove a spectrum from the Operands pane, click it and click **Remove**. To remove all the spectra, click **Clear All**.

   Note A spectrum appears in the Result pane only if two or more spectra are in the Operands pane.

3. To perform a math operation on the result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

   Note To use the result in another Average operation, use **Workbook** or **Temporary** to add it to the list (see step 4), click **Add** to make it an operand, and continue with step 2.

   Perform the operation as explained in the appropriate related topic below.

   To return to the previous window, click **Back**.

   To perform additional math operations, repeat this step.

4. To save the result in the current workbook, click **Workbook** and name the spectrum when prompted.

The data is added to the list and is available for operations.

To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

5. Click **Close** to close each displayed math window.

Related Topics

Math and Analysis Operations

Adding, Subtracting, Multiplying or Dividing Scan Data by a Factor

Use **Factor** in the Math menu to add a constant to the Y value of scan data, subtract a constant, multiply by a constant or divide by a constant.

❖ To use Factor

1. Choose **Math** (menu) > **Factor.**

   The selected spectrum appears in the Operands pane.

   The workbook spectra are listed at the left. To add others:

   a. Click **Browse**.

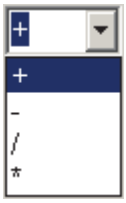
   b. Select a folder in the left pane (use the features above it to navigate), a workbook in the middle pane, and spectra in the upper-right pane. To select multiple spectra, hold down **Shift** or **Ctrl**. Selected spectra appear in the lower-right pane.

   c. Click **OK**. Each added spectrum is listed with asterisks at the beginning of its name to indicate the data is not in the current workbook.

2. Select any other listed spectra to operate on and click **Add**.

   To remove a spectrum from the Operands pane, click it and click **Remove**. To remove all the spectra, click **Clear All**.

3. Select the arithmetic operation to perform on the Y value of every data point.



4. Specify the factor: type a value, click the up and down arrow buttons, or drag the slider.

5. To perform a math operation on the result, click **Operate on Result** and choose an operation. Otherwise, go to the next step.

Note To use the result in another Factor operation, use **Workbook** or **Temporary** to add it to the list (see step 6), click **Add** to make it an operand, and continue with step 2.

Perform the operation as explained in the appropriate related topic below.

To return to the previous window, click **Back**.

To perform additional math operations, repeat this step.

6. To save the result in the current workbook, click **Workbook** and name the spectrum or spectra when prompted.

The data is added to the list and is available for operations.

To add the result to the list without saving it, click **Temporary**. Asterisks at the beginning of a spectrum name indicate the data is not saved.

7. Click **Close** to close each displayed math window.

Related Topics

Math and Analysis Operations

Finding Peaks in Scan Data

Use **Peak Pick** in the Analyze menu to find peaks, valleys, zero crossings, or maximum and minimum values in a spectrum or region.

Note Peaks can be found automatically after data acquisition. See Measurement Tab for Scan.

❖ To locate peaks

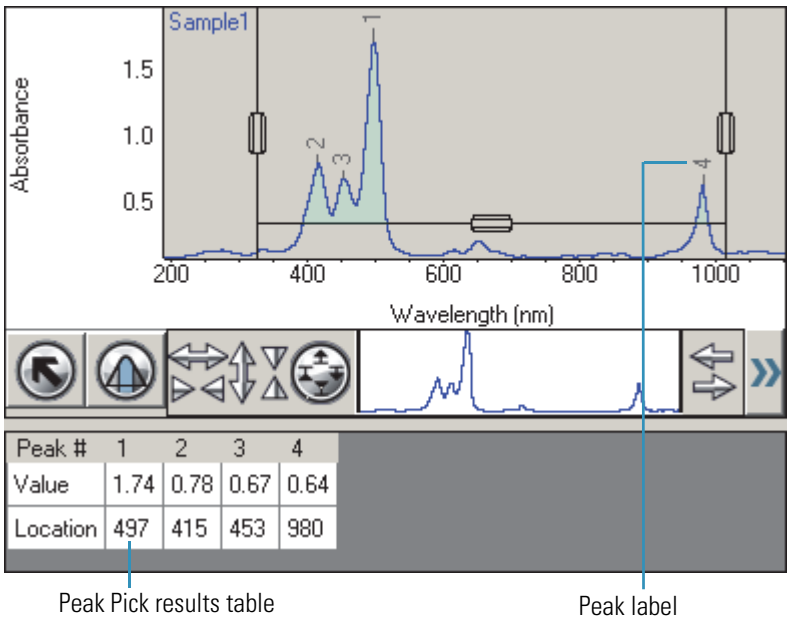
1. Choose **Analyze** (menu) > **Peak Pick**.
2. Set the parameters and adjust the data display as desired.

Parameter	Description
Find	Specifies whether to find peaks, valleys, both peaks and valleys, or maximum and minimum Y values.
Max number of peaks (or valleys, or peaks and valleys)	Maximum number of items to find.

Parameter	Description
Sort peaks (or valleys, or peaks and valleys) by	Order for labeling and listing found items in the Peak Pick results table.
Sensitivity	How readily small peaks or valleys are found. To optimize the sensitivity, select **Auto**. To set it manually, deselect **Auto** and drag the slider.
Wavelength Range	Wavelength range in which to find items. To use the entire range, select **Full**. To limit the range, deselect **Full** and type the desired limits or use the region-threshold tool: 
Use threshold	Specifies whether to find only those peaks whose Y values are greater than or equal to a specified value, the threshold. (For % transmittance, the software finds valleys less than or equal to the threshold.) To optimize the threshold value, select **Auto**. To set it manually, deselect **Auto** and set **Threshold** by typing a value or dragging the horizontal line in the data display.
Smooth spectrum	Specifies whether or not to smooth the data before finding peaks or valleys. This tends to reduce the number of small peaks, valleys and shoulders. (For more information, see About Smoothing.) The (blue) smoothed spectrum is overlaid on the original. To optimize the degree of smoothing, select **Auto**. To adjust it manually, deselect **Auto** and drag the slider.

To adjust the data display, use the view finder. To zoom in or move the spectrum, use the selection tool:

The results appear in the data display pane and the table below it:



3. Click **OK**.

   The found peaks have labels, and the peak results are listed at the right.

   The settings are saved in the workbook and will be used for measuring samples with Result on the Peak Pick tab set to Peak Pick.

Related Topics

Math and Analysis Operations

Finding Value Level Crossings in Scan Data

Use **Value Level Crossing** in the Analyze menu to find the wavelengths where a spectrum crosses a specified ordinate value.

Note Crossings can be found automatically after data acquisition. See Measurement Tab for Scan.

❖ To locate value level crossings

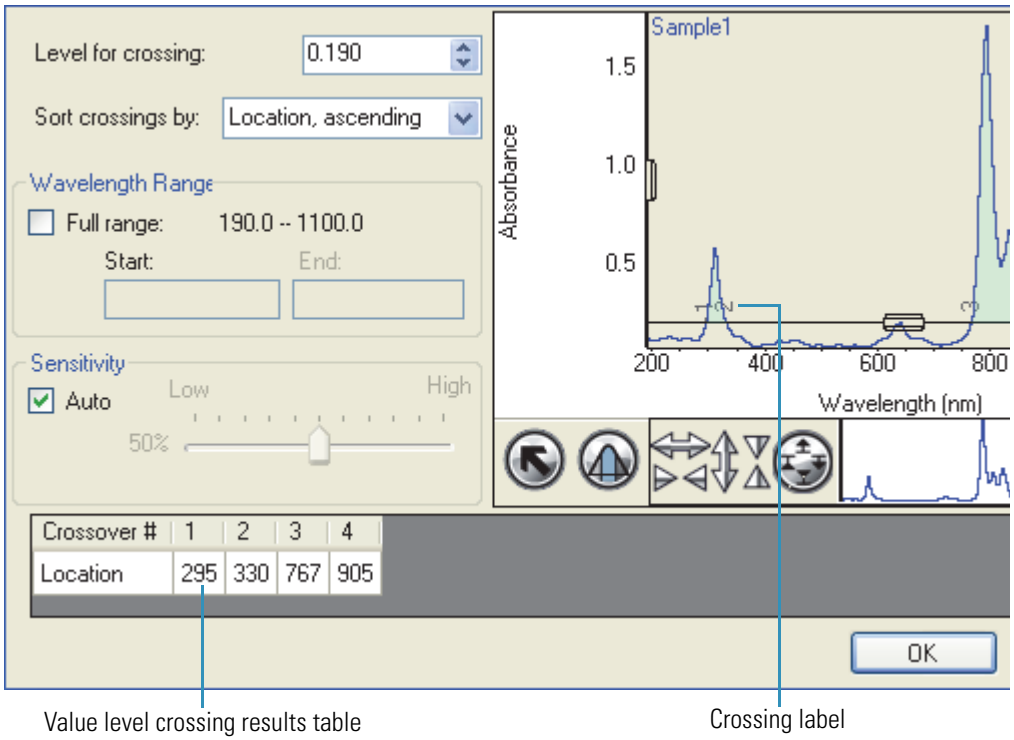
1. Choose **Analyze** (menu) **> Value Level Crossing**.
2. Set the parameters and adjust the data display as desired.

Parameter	Description
Level for crossing	Specifies the ordinate (Y) value. One can also drag the horizontal line in the data display.
Sort crossings by	Order for listing crossings in the value level crossings results table.

Parameter	Description
Wavelength Range	Wavelength range in which to find crossings. To use the entire range, select **Full**. To limit the range, deselect **Full** and type the desired limits or use the region-threshold tool: 
Sensitivity	How readily small-amplitude crossings are detected. To optimize the sensitivity, select **Auto**. To set it manually, deselect **Auto** and drag the slider. A high sensitivity finds locations where the spectrum crosses the horizontal line only a short distance.

To adjust the data display, use the view finder. To zoom in or move the spectrum, use the selection tool:

The results appear in the data display pane and the table below it:



3. Click **OK**.

The found crossings have labels, and the crossing results are listed at the right.

The settings are saved in the workbook and will be used for measuring samples with Result on the Peak Pick tab set to Value Level.

Related Topics

Math and Analysis Operations

Options

The following tabs are available in Options:

Applications Tab
Report Master Page Tab
Preferences Tab
Data Store Tab
Formulas and Units Tab

Applications Tab

Click **Options** to display the Applications tab in the right pane. Use it to **configure the home page** by adding new user groups and giving them access to particular applications. The Group drop-down list box shows the name of the current group.

❖ To add a new group

Enter the desired group name in the **Groups** box and click **Add**.

❖ To customize the current group

Drag the desired applications from the **Applications** list to the buttons at the right. To remove an application from the group, drag it from its button to the Applications list.

❖ To delete the current group

Click **Delete Group** and then click **Yes** when prompted.

❖ To reset the application buttons for the current group

Click **Clear App Buttons** and then click **Yes** when prompted.

❖ To make custom templates available for groups other than Classic

Drag the List of Templates application to one of the top nine menu buttons.

Report Master Page Tab

Click **Options** to display the Report Master Page tab in the right pane. Use it to define the appearance of all printed reports.

Feature	Description
Company name	Enter a company name and press **Enter**. Select an orientation for the name, and use **Font** to specify the font.
Date	Date of the report creation. Use **Font** to specify the font for Date and Time.
Time	Time of the report creation. Use **Font** to specify the font for Time and Date.
Logo	Select an orientation for the logo, and use **Browse** to locate and select a logo image file.
Footer text	Text at the bottom of the report. Use **Font** to specify the font for the footer.
Page number	Format for page numbers. Select **None** to not include page numbers.

Related Topics

Configuring a Report

Reports

Preferences Tab

Click **Options** to display the Preferences tab in the right pane.

Feature	Description
Data Value Digits	Specify the number of digits to the right of the decimal point to use for X and Y values for spectral data.
Triggering	Starting data acquisition normally requires a response to a prompt (displayed by Measure or another action button). To start acquisition automatically a specified number of minutes after the prompt appears, select **Enable auto input trigger** and enter the number of minutes for the delay. Use **Output trigger** to specify whether and when to produce a signal that triggers an installed accessory to begin an operation.

Feature	Description
Prevent removal of data	Prevents the removal of acquired data. When this option is selected, the following conditions are true: • **Remeasure** right-click option in the sample measurements table is enabled (if available). After a remeasurement, the previous information for the sample is crossed out. • **Remove** and **Remove all** (available for Scan applications only) are disabled. • Sample IDs cannot be edited in the sample measurements table below the data display and on the Samples tab in Reports.
Directories	The paths used for saving template files, Performance Verification reports, and workbooks. Type a path for each or click the button to specify a path.

Data Store Tab

Click **Options** to display the Data Store tab in the right pane. Use it to specify how to save workbooks, export data and e-mail results.

Feature	Description
Workbook Save Options	To be prompted to save data before acquiring it, select **Data saved on each measure**. Save Workbook and Save As Workbook will not be available in the File menu. To save acquired data only by using Save Workbook or Save As Workbook, select **Data saved only by Save Workbook in File menu**. (A prompt to save appears when INSIGHT software is closed if the current workbook has not been saved.)
Convert Data	Select **Auto export data** to automatically save acquired data using the selected format in the specified location. To change the location, type a new path or use **Browse**.
E-mail	Select **Auto e-mail result (workbook)** to automatically e-mail the current workbook. Enter the desired recipient address, subject and message.

Formulas and Units Tab

Click **Options** to display the Formulas and Units tab in the right pane. Use it to specify the default formulas, units and advanced equations for setting up quantitative analyses.

In the appropriate table, select in the **Show** column the items to be available.

To add an item, enter it (or the appropriate information) in the row that starts with a plus (+) sign.

To delete an item, right-click it and choose **Delete Row**. Items with a lock icon cannot be edited or deleted.

To return to the default selections, click **Reset**.

These formulas and units lists can be customized:

Feature	Description
Default formulas	These formulas appear when you click **Select** on the **Measurement tab** in **Quant Settings**.
Default units	These units appear in the **Unit** list box on the **Measurement tab** in **Quant Settings**.
Advanced user-defined calibration equations	These formulas appear when you click **Build** on the **Measurement tab** in **Quant Settings**.

Related Topics

Settings for Quant Applications

Performing a Quantitative Analysis

System Settings

These are available in System Settings:

Alignment Tab
Calibrations Tab
Lamp Tab
System Tab
Diagnostics tab (for our use only)

Alignment Tab

Click **System Settings** to display the Alignment tab in the right pane. Use it to view sample and reference detector intensities while aligning an accessory.

❖ To perform an alignment

1. To measure intensities using a green light (510 nm), select **Green**. To use no filter, select **White**.

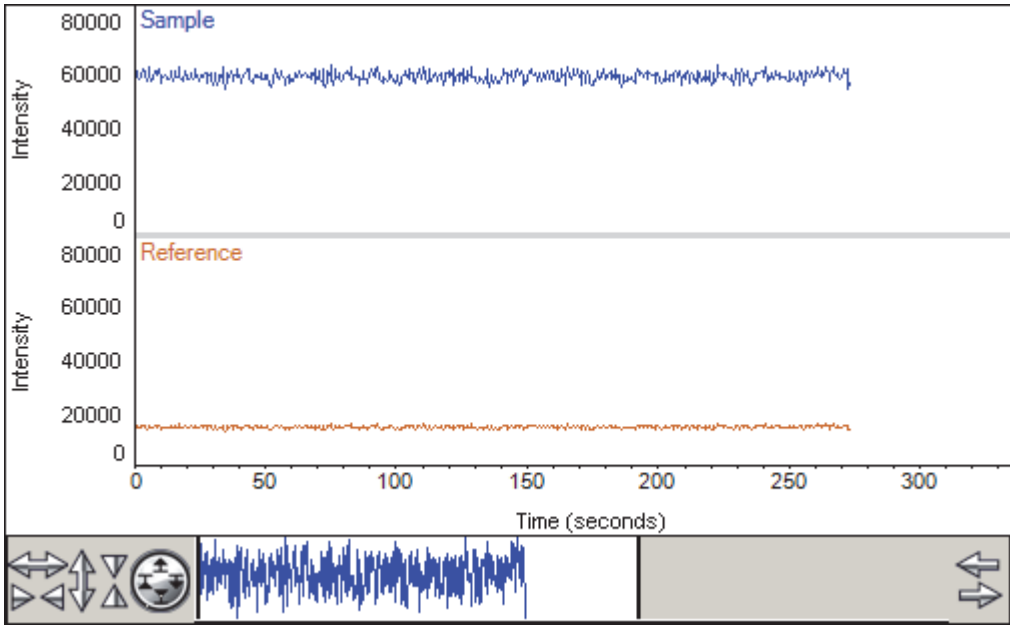
   The correct setting depends on the installed accessory. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

2. Set **Bandwidth** as desired.

   The available settings depend on the instrument.

3. Click **Start**.

   Every half second the sample and reference detector intensities are plotted and displayed by the Sample beam and Reference readouts.



   To overlay the plots instead of stacking them, deselect **Stack**.

> **Note** If the instrument has variable slit wheels, the start of intensity measurement is delayed while they move to their 2 nm position.

   The Time readout shows the elapsed time. Intensities can be measured and plotted for up to 15 minutes without restarting the process.

If the plot of sample detector intensity is not at the desired position for viewing, adjust the detector gain by dragging the **Gain** slider up or down.

To adjust the display, use the view finder. To zoom in, draw a box and click inside it. To return to full display, double-click the pane.

4. Align the accessory to achieve the desired sample detector intensity. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.
5. When finished, click **Stop**.

Related Topics

System Settings

Calibrations Tab

Click **System Settings** to display the Calibrations tab in the right pane. Use it to calibrate the instrument wavelength or accessories.

To calibrate using a particular lamp, click its **Calibrate** button and follow the instructions that appear. See Mercury Lamp for instructions for using a mercury lamp safely.

Related Topics

System Settings

Lamp Tab

Click **System Settings** to display the Lamp tab in the right pane. Use it to display status information about the xenon lamp.

Related Topics

System Settings

System Tab

Click **System Settings** to display the System tab in the right pane. Use it to switch the instrument between local and computer control and to update firmware.

Feature	Description
Instrument Control	Switches instrument control between the Local Control option and INSIGHT software running on an external computer. Select the desired setting and respond appropriately to the message that appears.
Firmware Update	Click **Load Update** to install the selected instrument firmware. Follow the instructions that appear.
Instrument Boot Record	Click **Display** to view the instrument's power-up boot record, which can be saved or copied.

Related Topics

System Settings

Keyboard Shortcuts

These are available in INSIGHT software:

Keyboard Shortcut	Description
Alt+F4	Exits INSIGHT software.
Ctrl+F (from Home)	Displays Fixed.
Ctrl+Q (from Home)	Displays Quant.
Ctrl+R (from Home)	Displays Rate.
F1	Displays Help.
F2	Displays Home.
F4	Displays the Measure screen for the current application.
F5	Acquires zero or baseline data.
F6	Chooses the Measure button.
F7	Prints the Measure screen.
F8	Prints the report.
F9	Displays My Data.
F10	Creates a new workbook.
F11 (from Home)	Displays Performance Verification.
F12	Chooses the Stop button.

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INSIGHT Bio Applications

Contents

About the Bio Applications

The Bio applications provide methods for measuring biological macromolecules including nucleic acids and proteins. The application type, settings and report layout for each Bio application are pre-configured but customizable. If you customize one of these applications, you can save the current settings as the default settings or save them in a workbook.

Nucleic Acid Assays

The nucleic acid assay methods include:

- Nucleic Acid
- Nucleic Acid Labels
- DNA Melting

Below are the default values for the nucleic acid methods. Wavelengths are in nanometers.

Method	Analytical Wavelength (Quant)	Reference Wavelength	Start (Scan)	Stop (Scan)	Bandwidth (nm)	Integration Time (sec) (Fixed, Scan)	Data Interval (Scan)	Scan Speed (nm/min)
Nucleic Acid	260	340	220	350	1.0	1.0, 0.08	0.50	375
Nucleic Acid Labels	260	340	220	850	1.0	1.0, 0.16	1.00	375
DNA Melting	260	340	n/a	n/a	1.0	1.0	n/a	n/a

Protein Assays

There are two types of protein assay methods included, direct UV and colorimetric assays. The colorimetric assays include several assays that are similar. Likewise, the direct protein methods are also very similar. Use the table below to find the appropriate assay procedure. Click the links to access complete information about the assays.

Direct UV Assays	Colorimetric Assays
Protein A280	Protein A280
Proteins and Labels	Protein Bradford
	Pierce BCA
	Pierce Modified Lowry
	Pierce 660 nm Protein Assay
	Protein Biuret

Below are the default values for the protein methods. Wavelengths are in nanometers.

Method	Analytical Wavelength (Quant)	Reference Wavelength	Start (Scan)	Stop (Scan)	Bandwidth	Integration Time (sec) (Fixed, Scan)	Data Interval (Scan)	Scan Speed (nm/min)
Protein A280		340	220	850	1.0	1.0, 0.08	0.5	375
Proteins & Labels		340	220	850	1.0	1.0, 0.16	1.0	375
Pierce BCA	562	340	750	450	1.0	1.0, 0.12	1.00	500
Protein Bradford	595	340	750	450	1.0	1.0, 0.12	1.00	500
Pierce Modified Lowry	750	405	900	600	1.0	1.5, 0.12	1.00	500
Pierce 660 nm Protein Assay	660	340	850	550	1.0	1.5, 0.12	1.00	500
Protein Biuret	550	340	750	450	1.0	1.0, 0.12	1.00	500

Nucleic Acid

Contents

Overview

Use the Nucleic Acid application to measure nucleic acid samples for concentration and purity.

See Unique Screen Features for information about the results the application calculates. See Nucleic Acid Concentration Calculations for information about the calculations.

Related Topics

Nucleic Acid Concentration Calculations

Application Settings

Data Display

Unique Screen Features

Making Nucleic Acid Measurements

Oligo Calculator

Nucleic Acid Concentration Calculations

The Nucleic Acid application is used for determining the concentration and purity of nucleic acid samples. The nucleic acid concentration is determined by multiplying the absorbance at 260 nm by a factor (default, 50). This factor is essentially the extinction coefficient in units of ng-cm/ml. Using this coefficient gives the manipulated equation

$$c = (A * \varepsilon)/\ell$$

where c is the nucleic acid concentration in ng/microliter, A is the absorbance in AU, ε is the wavelength-dependent extinction coefficient in ng-cm/microliter, and ℓ is the pathlength in centimeters.

The generally accepted extinction coefficients for nucleic acids are:

Coefficient	Value
Double-stranded DNA	50 ng-cm/?l
Single-stranded DNA	33 ng-cm/?l
RNA	40 ng-cm/?l

Related Topics

Overview

Application Settings

Data Display

Unique Screen Features

Making Nucleic Acid Measurements

Oligo Calculator

Nucleic Acid Purity Measurements

The 260/280 nm ratio assay determines the purity of nucleic acids based on the 260/280 ratio.

The 260/230 ratio assay determines the purity of nucleic acids in the presence of phenol, which absorbs strongly at 230 nm.

A ratio of approximately 1.8 is generally accepted as "pure" for DNA, approximately 2.0 for RNA. If either ratio is appreciably lower, protein, phenol or other contaminants that absorb strongly near 280 nm may be present.

The 260/230 values for a "pure" nucleic acid are often higher than the respective 260/280 values and are commonly in the range 1.8 to 2.2. If the ratio is appreciably lower, co-purified contaminants may be present.

Application Settings

To set data acquisition parameters for a Nucleic Acid workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Nucleic Acid
Measurement Tab for Nucleic Acid
Instrument Tab for Nucleic Acid
Accessories Tab for Nucleic Acid
Samples Tab for Nucleic Acid

Related Topics

Overview

Nucleic Acid Concentration Calculations

Data Display

Unique Screen Features

Making Nucleic Acid Measurements

Oligo Calculator

Type Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Type tab in the right pane.

These settings are available:

Feature	Description
Name	Application name.
Description (optional)	Description of the template.
Nucleic Acid Type	Type of analysis to perform. ssDNA is for single-stranded DNA. Oligo DNA and Oligo RNA use the appropriate extinction coefficient based on entered DNA or RNA base sequence. The entry will appear on the Oligo Calculator tab. For Custom, enter a factor (extinction coefficient) by which to multiply the measured absorbance. The factor used for other types cannot be changed.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Nucleic Acid

Instrument Tab for Nucleic Acid

Accessories Tab for Nucleic Acid

Samples Tab for Nucleic Acid

Measurement Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Measurement tab in the right pane.

These settings are available:

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Nucleic Acid

Instrument Tab for Nucleic Acid

Accessories Tab for Nucleic Acid

Samples Tab for Nucleic Acid

Instrument Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Instrument tab in the right pane.

These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Nucleic Acid

Measurement Tab for Nucleic Acid

Accessories Tab for Nucleic Acid

Samples Tab for Nucleic Acid

Accessories Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Nucleic Acid

Measurement Tab for Nucleic Acid

Instrument Tab for Nucleic Acid

Samples Tab for Nucleic Acid

Samples Tab for Nucleic Acid

Click **Settings** in Nucleic Acid to display the Samples tab in the right pane.

These settings are available:

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.

Feature	Description
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Nucleic Acid

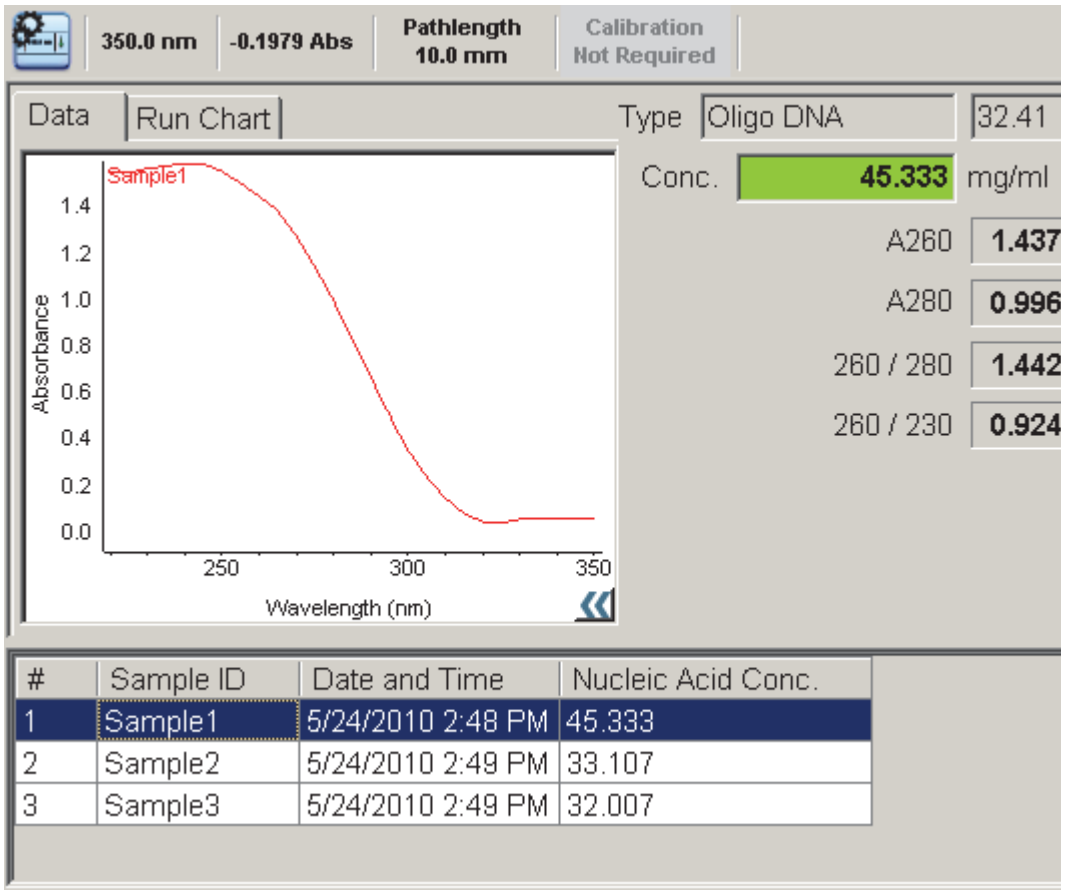
Measurement Tab for Nucleic Acid

Instrument Tab for Nucleic Acid

Accessories Tab for Nucleic Acid

Unique Screen Features

The right pane in Measure Nucleic Acid displays workbook parameters specific to the application. Task buttons in the left pane are described in Task Buttons.



The spectral display shows data for the current sample.

These features are to the right of the spectral display:

Feature	Description
Type	Type of analysis to perform. To the right is the factor value from the Type tab.
Conc.	Concentration determined by multiplying the absorbance at 260 nm by a factor (default, 50) after correction is applied. Beer's law is used to calculate the nucleic acid concentration (see Nucleic Acid Concentration Calculations).
A260	Absorbance at 260 nm.
A280	Absorbance at 280 nm.

Feature	Description
260/280	Ratio of sample absorbance at 260 nm and 280 nm, used to assess the purity of DNA and RNA. A ratio of about 1.8 is generally accepted as "pure" for DNA, about 2.0 for RNA. If either ratio is appreciably lower, protein, phenol or other contaminants that absorb strongly near 280 nm may be present. See Nucleic Acid Purity Measurements for more information.
260/230	Ratio of absorbance at 260 nm and 230 nm, a secondary measure of nucleic acid purity.

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.

4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).
7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.
9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Nucleic Acid Concentration Calculations

Making Nucleic Acid Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

❖ **To measure nucleic acids**

1. Click **Measure Nucleic Acid** in Nucleic Acid.

Note To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. Click **Measure**.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank, generally the same buffer used to prepare or dissolve the sample. The buffer should be the same pH and of a similar ionic strength as the sample solution.

4. When samples information appears, modify it if desired.

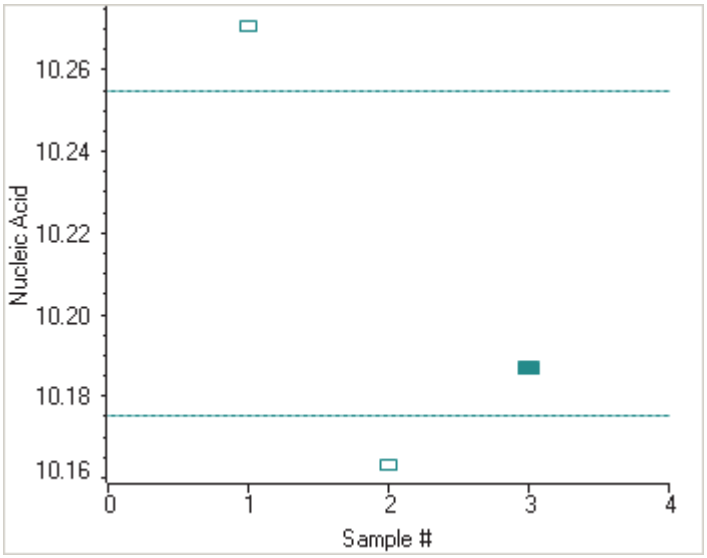
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

5. If only one sample will be measured, insert it.
6. Click **Continue**.
7. Follow any instructions that appear, such as to install a specified sample.

The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Related Topics

Overview

Nucleic Acid Concentration Calculations

Application Settings

Data Display

Unique Screen Features

Oligo Calculator

Oligo Calculator

Use **Oligo Calculator** to calculate molecular weight, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Clicking this task button displays:

Tab	Description
Oligo Calculator	For entering sequences of interest and selecting appropriate sample type variables.
Melting	Displays the calculated melting points of a DNA strand. Available only for DNA sequences.

❖ To use Oligo Calculator

1. Enter a base sequence on the Oligo Calculator tab.

   More:

   Use:

   - The buttons below the Base Sequence box.
   - The A, C, G, T and U keys on the keyboard.
   - Copy and paste a base sequence (A, C, G, T and U only) from another application.

   To clear the sequence, click **Clear**. Individual bases can be deleted only manually.

2. Select the nucleic acid type to analyze.
3. Select the degree of phosphorylation if applicable: mono-phosphate for DNA; mono- or tri-phosphate for RNA.
4. Select **Double-stranded** if applicable. The complementary base sequence will be included in the analysis.
5. Enter the molecular weights of any additions to the base sequence in the **Modification** box.

   The calculated results appear:

Result	Description
Molecular weight	Calculated base sequence molecular weight.
Molar extinction coefficient	Calculated 260 nm extinction coefficient in ng-cm/microliter.
Concentration factor	Factor, based on the extinction coefficient, used to calculate the concentration of the base sequence.

Result	Description
Number of bases	Number of bases in the entered sequence.
% GC	Percentage of the total number of bases made up by guanine and cytosine.

❖ **To calculate the melting point of a DNA sequence**

1. Enter the base sequence on the Oligo Calculator tab.
2. Enter appropriate values on the Melting tab:

Parameter	Description
Oligo molarity	Concentration of the Oligo in molar units.
Cation molarity	Concentration of cations in the sample.
% Formamide	Percentage concentration of formamide in the sample.

The calculated results appear:

Result	Description
Salt-adjusted	Calculated melting point of the base sequence, corrected for the concentration of cation in the sample. This method does not account for the effect of interaction between neighboring bases.
Nearest-neighbor	Melting point of the base sequence when the effect of interaction between neighboring bases is taken into account.

Related Topics

Overview

Nucleic Acid Concentration Calculations

Application Settings

Data Display

Unique Screen Features

Making Nucleic Acid Measurements

Nucleic Acid Labels

Contents

Overview

Use the Nucleic Acid Labels application to measure nucleic acid samples for concentration and purity.

Related Topics

Dye/Chromophore Editor

Application Settings

Data Display

Unique Screen Features

Making Nucleic Acid Labels Measurements

Oligo Calculator

Dye/Chromophore Editor

Use the Dye Chromophore Editor to select predefined dyes, modify existing dyes for protocols, or enter new dyes.

To enter a new dye, type its information in the appropriate cells of the bottom row of the table and select a unit. Refer to the dye manufacturer for appropriate correction factors. The 260 nm corrections will be used for nucleic acid sample concentration calculations. Entered information is saved automatically.

To delete a user-defined dye, right-click its row and choose **Delete Row**. Predefined dyes, identified by the lock icon, cannot be edited or deleted.

Related Topics

Overview

Application Settings

Data Display

Unique Screen Features

Making Nucleic Acid Labels Measurements

Oligo Calculator

Application Settings

To set data acquisition parameters for a Nucleic Acid Labels workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Nucleic Acid Labels
Measurement Tab for Nucleic Acid Labels
Instrument Tab for Nucleic Acid Labels
Accessories Tab for Nucleic Acid Labels
Samples Tab for Nucleic Acid Labels

Related Topics

Overview

Dye/Chromophore Editor

Data Display

Unique Screen Features

Making Nucleic Acid Labels Measurements

Oligo Calculator

Type Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Type tab in the right pane.

These settings are available:

Feature	Description
Name	Application name.
Description (optional)	Description of the template.
Nucleic Acid Type	Type of analysis to perform. ssDNA is for single-stranded DNA. Oligo DNA and Oligo RNA use the appropriate extinction coefficient based on the entered DNA or RNA base sequence. The entry will appear on the Oligo Calculator tab. For Custom, enter a factor (extinction coefficient) by which to multiply the measured absorbance. The factor used for other types cannot be changed. Set Dye 1 and Dye 2 to the fluorescent dyes whose absorbance will be used in concentration correction calculations.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Nucleic Acid Labels

Instrument Tab for Nucleic Acid Labels

Accessories Tab for Nucleic Acid Labels

Samples Tab for Nucleic Acid Labels

Measurement Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Measurement tab in the right pane.

These settings are available:

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Nucleic Acid Labels

Instrument Tab for Nucleic Acid Labels

Accessories Tab for Nucleic Acid Labels

Samples Tab for Nucleic Acid Labels

Instrument Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Instrument tab in the right pane.

These settings are available:

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Nucleic Acid Labels

Measurement Tab for Nucleic Acid Labels

Accessories Tab for Nucleic Acid Labels

Samples Tab for Nucleic Acid Labels

Accessories Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Nucleic Acid Labels

Measurement Tab for Nucleic Acid Labels

Instrument Tab for Nucleic Acid Labels

Samples Tab for Nucleic Acid Labels

Samples Tab for Nucleic Acid Labels

Click **Settings** in Nucleic Acid Labels to display the Samples tab in the right pane.

These settings are available:

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.

Feature	Description
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Nucleic Acid Labels

Measurement Tab for Nucleic Acid Labels

Instrument Tab for Nucleic Acid Labels

Accessories Tab for Nucleic Acid Labels

Unique Screen Features

The right pane in Measure Nucleic Acid Labels includes:

Feature	Description
Type	Type of analysis to perform. To the right is the factor value from the Type tab.
Conc.	Concentration based on absorbance at 260 nm, the default or user-defined extinction coefficient, and the specified pathlength. (The extinction coefficient is reported for a 10 mm pathlength.) Beer's law is used to calculate the nucleic acid concentration (see Nucleic Acid Concentration Calculations). The displayed A260 value is baseline corrected. The A260 value actually used to calculate the concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction wavelength.

Feature	Description
A260	Absorbance at 260 nm.
260/280	Ratio of sample absorbance at 260 nm and 280 nm, used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA, ~2.0 for RNA. If either ratio is appreciably lower, protein, phenol or other contaminants that absorb strongly near 280 nm may be present. See Nucleic Acid Purity Measurements for more information.
Dye(s) Conc.	Concentration of the user-selected dye(s). The software automatically subtracts the value of a sloping baseline from 400 to 750 nm from the absorbance at the Dye wavelength. Only the corrected absorbance of the dye peak(s) and dye concentration(s) are reported.

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.

2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.
4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).
7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.
9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Dye/Chromophore Editor

Application Settings

Data Display

Making Nucleic Acid Labels Measurements

Oligo Calculator

Making Nucleic Acid Labels Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

❖ **To make a Nucleic Acid Labels measurement**

1. Click **Measure Nucleic Acid Labels** in Nucleic Acid Labels.

Note To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. Click **Measure**.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank, generally the same buffer used to prepare or dissolve the sample. The buffer should be the same pH and of a similar ionic strength as the sample solution.

4. When samples information appears, modify it if desired.

More:

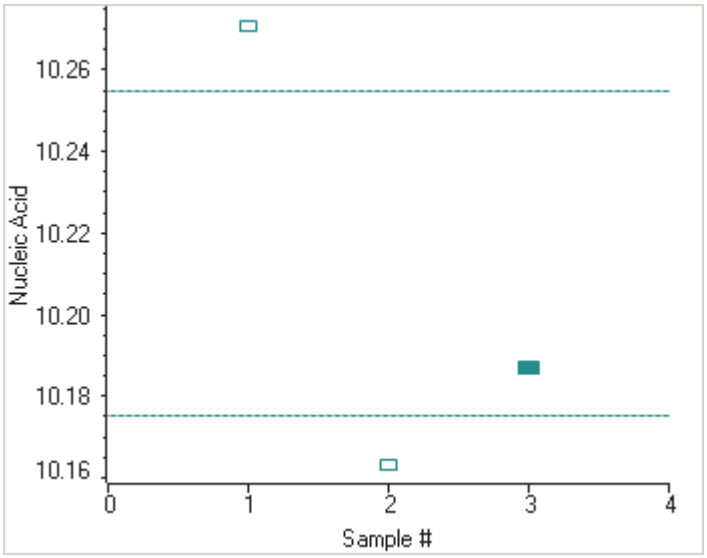
If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

5. If only one sample will be measured, insert it.
6. Click **Continue**.
7. Follow any instructions that appear, such as to install a specified sample.

Note Use a fresh aliquot of sample for each measurement.

The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Related Topics

Overview

Dye/Chromophore Editor

Application Settings

Data Display

Unique Screen Features

Oligo Calculator

After the Measurement

- When using the cuvette option, remove the cuvette, rinse thoroughly and dry between samples.

Oligo Calculator

Use **Oligo Calculator** to calculate molecular weight, extinction coefficients, concentration factors and melting points for specific nucleic acid sequences. Clicking this task button displays:

Tab	Description
Oligo Calculator	For entering sequences of interest and selecting appropriate sample type variables.
Melting	Displays the calculated melting points of a DNA strand. Available only for DNA sequences.

❖ To use Oligo Calculator

1. Enter a base sequence on the Oligo Calculator tab.

   More:

   Use:

   - The buttons below the Base Sequence box.
   - The A, C, G, T and U keys on the keyboard.
   - Copy and paste a base sequence (A, C, G, T and U only) from another application.

   To clear the sequence, click **Clear**. Individual bases can be deleted only manually.

2. Select the nucleic acid type to analyze.
3. Select the degree of phosphorylation if applicable: mono-phosphate for DNA; mono- or tri-phosphate for RNA.
4. Select **Double-stranded** if applicable. The complementary base sequence will be included in the analysis.
5. Enter the molecular weights of any additions to the base sequence in the **Modification** box.

The calculated results appear:

Result	Description
Molecular weight	Calculated base sequence molecular weight.
Molar extinction coefficient	Calculated 260 nm extinction coefficient in ng-cm/microliter.
Concentration factor	Factor, based on the extinction coefficient, used to calculate the concentration of the base sequence.
Number of bases	Number of bases in the entered sequence.
% GC	Percentage of the total number of bases made up by guanine and cytosine.

❖ To calculate the melting point of a DNA sequence

1. Enter the base sequence on the Oligo Calculator tab.
2. Enter appropriate values on the Melting tab:

Parameter	Description
Oligo molarity	Concentration of the Oligo in molar units.
Cation molarity	Concentration of cations in the sample.
% Formamide	Percentage concentration of formamide in the sample.

The calculated results appear:

Result	Description
Salt-adjusted	Calculated melting point of the base sequence, corrected for the concentration of cation in the sample. This method does not account for the effect of interaction between neighboring bases.
Nearest-neighbor	Melting point of the base sequence when the effect of interaction between neighboring bases is taken into account.

Related Topics

Overview

Dye/Chromophore Editor

Application Settings

Data Display

Unique Screen Features

Making Nucleic Acid Labels Measurements

DNA Melting

Contents

Overview

Use the DNA Melting application to determine the temperature at which a DNA sample denatures based on the change in absorbance as the temperature is increased.

As the temperature is increased, the electronic interactions between the DNA bases are modified and the entire absorption spectrum increases in intensity. This phenomenon is called the hyperchromic effect and the increase in absorption due to the abrupt change in structure is usually around 30–40%.

See Unique Screen Features for information about the DNA Melting data acquisition results. For information about the calculations, see DNA Melting Point Calculations.

DNA Melting Point Calculations

The melting of DNA occurs over a narrow temperature range. The midpoint of this transition is called the melting temperature or T_m. The melting temperature depends strongly on the percentage of the stronger hydrogen bonding G-C (guanine-cytosine) base pairs present in the DNA sequence. The T_m value for a particular DNA molecule will be reproducible as long as the pH, ionic strength, and buffer conditions do not change. Subtle changes to any of these solution conditions can alter the T_m value.

The T_m value of a DNA molecule can be calculated three ways:

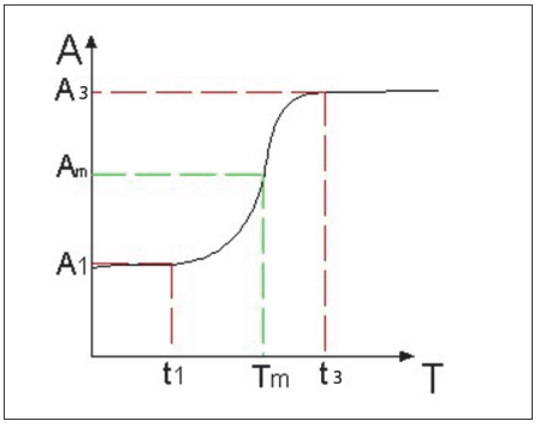
- **Horizontal Intercept**. Useful when absorbance does not vary with temperature outside the melting range. Requires two temperature values (t_1 and t_3).

  T_1 is a stable temperature value in °C that occurs below the temperature range in which the DNA melting point occurs. A_1 is the absorbance at T_1.

  T_3 is a stable temperature value in °C that occurs above the DNA melting range. A_3 is the absorbance at T_3.

  A_m is a calculated absorbance that is midway between A_1 and A_3.

T_m is the temperature at A_m.



- **Sloping Intercept**. Used when the absorbance varies with temperature inside and outside the melting range. Requires three temperature values (t_1, t_2 and t_3).

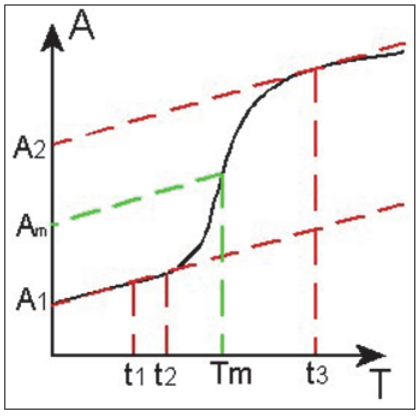
  T_1 is a temperature value in °C that occurs below the temperature range in which the DNA melting point occurs.

  T_3 is a temperature value in °C that occurs above the DNA melting range.

  T_2 is an intermediate temperature value in °C.

  The software constructs a best fit line between the point at T_2 and the point at the other temperature that is closest to it. A second line is drawn through the third point and parallel with the first line. A_m (the absorbance at T_m) is midway between the points where the two parallel lines intercept the Y-axis.

  A third line parallel to the others is drawn from A_m to the Absorbance vs Temperature curve, and a perpendicular line is dropped from that intersection to the X-axis. T_m is the temperature at which the perpendicular line intercepts the X-axis.



- **Inflection**. Calculates the first derivative of the Absorbance vs Temperature curve. T_m is the temperature at which the first derivative curve has its maximum value (i.e., the slope). Requires two temperature values (t_1 and t_3), which define the range over which to perform the fit.

Related Topics

Overview

Application Settings

Unique Screen Features

Making DNA Melting Measurements

Application Settings

To set data acquisition parameters for a DNA Melting workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for DNA Melting
Measurement Tab for DNA Melting
Instrument Tab for DNA Melting
Accessories Tab for DNA Melting
Samples Tab for DNA Melting
Tm Calculations Tab for DNA Melting

Related Topics

Overview

DNA Melting Point Calculations

Unique Screen Features

Making DNA Melting Measurements

Type Tab for DNA Melting

Click **Settings** in DNA Melting to display the Type tab in the right pane.

These settings are available:

Feature	Description
Name (if present)	User group name followed by the name of the template of settings (entered when the template was saved using Save Workbook Settings as Template in the File menu).
Description	Description of the template.

Feature	Description
Rate Type	Type of rate measurement to perform (see the table below). An image representing the selected type appears to the right. The required tabs also appear (click the Reset action button to step through them). These options are available: • **Single wavelength**. For each sample, measures a specified wavelength at specified intervals of time. • **Multiple wavelengths**. For each sample, measures multiple specified wavelengths at specified intervals of time. Up to 40 wavelengths can be specified.
Mode	Temperature is the only available option for the DNA Melting application. It performs rate measurements based on elapsed time and also records temperature settings.

Related Topics

Overview

DNA Melting Point Calculations

Unique Screen Features

Making DNA Melting Measurements

Measurement Tab for DNA Melting

Click **Settings** in DNA Melting to display the Measurement tab in the right pane.

These settings are available:

Feature	Description
Integration time	How long the system acquires and averages data to obtain each data point at each measured wavelength for single and multicell experiments.
Dwell time	How long the sample remains in the light beam. Available for single and multicell experiments. Use this setting to vary the number of data points collected per cell per cycle. Dwell time is always a multiple of integration time (see above). Dwell time can be very useful when performing multicell rate measurements. This is because the cell changer accessory will position a cell in the light beam for a specified period of time (dwell time), rather than moving back and forth between cells and increasing the time between measurements (when no sample is in the light beam). For example, if the integration time is 0.1 second and dwell time is 0.5 second, five data points will be recorded for each cell before the cell changer moves to the next cell. For fairly fast reactions, multiple samples can be measured, with over ten times as many data points acquired within a short time. This can improve the accuracy of rate calculations for faster multicell rate measurements.
Number of stages	Defines the number of measurement cycles over which the frequency of data acquisition can be determined. For example, if a reaction has two components, the first component could be fast and require a high data density. The second component could be much slower and longer lived, therefore requiring a lower data density. Defining multiple stages enables the use of the proper data density over a given period of time.
Temp unit	Determines the temperature unit used in the stages table (see below).

Feature	Description
Time unit	Determines the time unit used in the stages table (see below).
Stages table	Defines data acquisition for each stage of an experiment including the target temperature, ramp rate, hold type, hold time, interval and whether data collection will occur. Note that the temperature in the final stage can be set to a value around 10 °C to simulate storage in a refrigerator. This feature allows proper storage conditions for the sample if DNA melting experiments will be completed when the instrument is unattended. **Target Temp**. Enter a target temperature between 0.00 °C (32.00 °F or 273.15 °K) and 110.00 °C (230.00 °F or 383.15 °K). **Ramp Rate**. Enter a ramp rate from 0.40 to 20.00 °C/min (0.72 to 36.00 °F/min or 0.40 to 20.00 °K/min) up or down. **Hold Type**. Specify a pause at the target temperature before continuing to the next stage. **Time** holds for a specified time (see Hold Time below). **Prompt** holds until the operator responds to a message in the software. **Trigger** waits for an external trigger. Choosing Start in the trigger prompt will override the trigger. **Hold Time**. When Hold Type is set to Time, enter the length of time to hold at the target temperature before continuing to the next stage. **Interval**. Specifies how frequently a measurement is made. Must be equal to or greater than the specified Integration Time and less than the stage duration. Disabled when **Collect Data** (see below) is set to No. **Collect Data**. Specifies whether to collect data during each stage.

Related Topics

Overview

DNA Melting Point Calculations

Unique Screen Features

Making DNA Melting Measurements

Instrument Tab for DNA Melting

Click **Settings** in DNA Melting to display the Instrument tab in the right pane.

These settings are available:

Feature	Description
Data mode	Y-axis format for acquired data. Absorbance is the only available option for the DNA Melting application.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Reference wavelength correction	When selected, automatically acquires a reference measurement at a specified wavelength each time a sample measurement is taken. The reference measurement is then subtracted from the corresponding sample measurement to produce a corrected measurement result. Only the corrected result is reported. Use the **Reference wavelength** box to specify a wavelength for the reference measurement.
Wavelength Summary	Wavelengths to monitor during the reaction. DNA Melting is typically performed at 260 nm for single wavelength measurements and at 260 nm and 280 nm for multi-wavelength experiments.

Related Topics

Overview

DNA Melting Point Calculations

Unique Screen Features

Making DNA Melting Measurements

Accessories Tab for DNA Melting

Click **Settings** in DNA Melting to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Evolution 200 Series Accessories

Instrument Status Monitors

Overview

DNA Melting Point Calculations

Unique Screen Features

Making DNA Melting Measurements

Samples Tab for DNA Melting

Click **Settings** in DNA Melting to display the Samples tab in the right pane.

These settings are available:

Feature	Description
Number of samples	Number of samples for the experiment.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name “dye” would be dye1, dye2 and dye3.
Set up sample table columns	Specifies the columns of sample information to be entered during the experiment. To add a column, type the desired name in an empty cell and press the Tab key. To delete a column (except the Sample ID column), right-click it and choose **Delete Column**. To delete all the columns (except the Sample ID column), right-click the table and choose **Delete All Columns**.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table on the Samples tab.

Feature	Description
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. Additional columns are defined by the **Set up sample table columns** option (see above). To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Overview

DNA Melting Point Calculations

Unique Screen Features

Making DNA Melting Measurements

Tm Calculations Tab for DNA Melting

Click **Settings** in DNA Melting to display the T_m Calculations tab in the right pane.

These settings are available:

Feature	Description
Calculate T_m values automatically at end of data acquisition	Calculates DNA melting point temperature (T_m) values automatically at the end of the experiment. When this option is selected, the entered temperature values are marked in the data display and the calculated T_m values appear in the sample measurements table after the measurement is completed. To modify the T_m settings, **double-click the data display** or choose **Analyze (menu) > T_m Calculations**. When this option is cleared and T_m calculations have been entered, use the Modify Tm Calculations window to view, modify, update and accept the T_m calculation settings. After you accept the settings, the window closes and the temperature values are marked in the data display and the T_m values appear in the sample measurements table. See Making DNA Melting Measurements for more information.

Feature	Description
T_m Calculation	Select a method for calculating DNA melting point temperature values. These options are available: • **Horizontal Intercept**. Uses the absorbance at two specified temperature values and their horizontal intercepts to calculate T_m. Useful when absorbance does not vary with temperature outside the melting range. • **Sloping Intercept**. Uses the absorbance at three specified temperature values and their sloped intercepts to calculate T_m. Select this option when the absorbance varies with temperature inside and outside the melting range. • **Inflection**. Calculates the first derivative of the Absorbance vs Temperature curve. T_m is the temperature at which the first derivative curve has its maximum value (i.e., the slope). For more information, see DNA Melting Point Calculations.
Temperature 1, 2, 3	Enter the temperatures required by the calculation method. • **Horizontal Intercept**. In the Temperature 1 box, enter a temperature value in °C from a horizontal section of the graph that occurs below the temperature range in which the DNA melting point occurs. In the Temperature 3 box, enter a temperature value in °C from a horizontal section of the graph that occurs above the DNA melting range. • **Sloping Intercept**. In the Temperature 1 box, enter a temperature value in °C that occurs below the temperature range in which the DNA melting point occurs. (Choose a point where the slope is typical.) In the Temperature 3 box, enter a temperature value in °C that occurs above the DNA melting range. (Again, choose a point where the slope is typical.) In the Temperature 2 box, enter an intermediate temperature value in °C. (Choose a point that is close to t_1 or t_3). • **Inflection**. In the Temperature 1 box, enter a temperature value in °C that defines the start of the range used to calculate the derivative curve. In the Temperature 3 box, enter the temperature value in °C that defines the end of the calculation range.

Related Topics

Overview

DNA Melting Point Calculations

Unique Screen Features

Making DNA Melting Measurements

Unique Screen Features

Task buttons in the left pane are described in Task Buttons. The right pane in DNA Melting is described in Data Display.

These features are to the left of the spectral display:

Feature	Description
Extend Time	To extend the collection time for the current sample measurement (without interrupting the data collection), click **Extend Time** and enter the amount of time to extend the measurement. If you are collecting data in multiple stages, only the current stage is affected.

Related Topics

Overview

DNA Melting Point Calculations

Application Settings

Unique Screen Features

Making DNA Melting Measurements

Data Display

Making DNA Melting Measurements

DNA Melting measurements can be made at one wavelength or at up to 40 wavelengths in the same experiment. This includes multicell measurements, which allows running a fixed-wavelength measurement on several samples simultaneously using a sample changer.

Measurements are taken at specified intervals of time for each stage defined on the Measurements tab in Settings. Temperature settings and ramp rate are controlled and recorded for the duration of the experiment.

It may be helpful to display the following status monitors during your DNA Melting experiments:

- Cell Changer status
- Temperature Controller status
- Time status
- Temperature probe status

To display status monitors, click the **Configure Instrument Status** icon above the data display.

In the Configure Instrument Status box, select the status monitors to display and click **OK**.

Note To configure the software to display status monitors automatically, display the status monitors and then save the workbook as a template.

❖ To measure the melting point of a DNA sample

1. Configure the DNA Melting settings as desired.
2. Click **Measure**.

3. Follow the instructions that appear.
4. When samples information appears, modify it if desired.

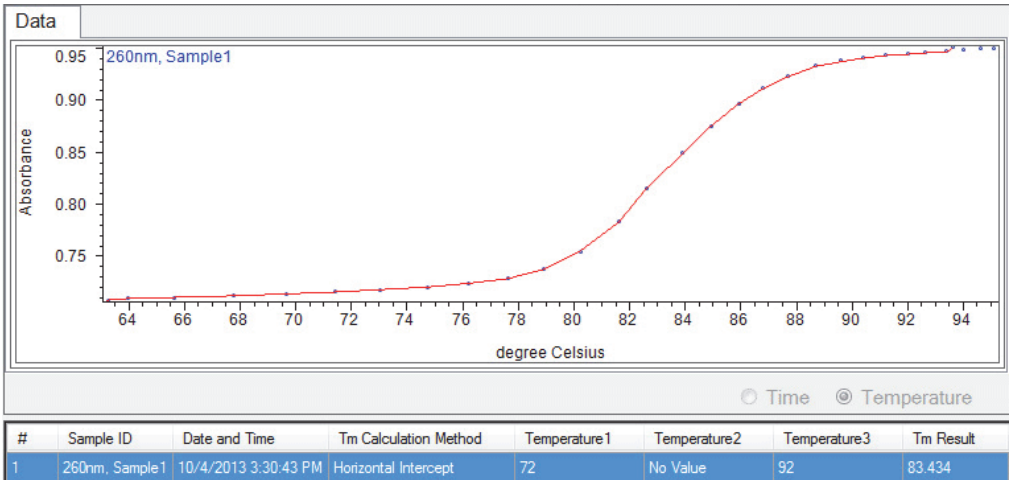
   To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

   Enter values in any special columns that were specified on the Samples tab in Settings. These values may appear in the DNA Melting data report.

5. Click **Continue**.
6. Follow the instructions that appear.

   For each measured sample, the Data Display shows an absorption spectrum with the Y-axis in absorbance and the X-axis in temperature. Each plot shows a sample's spectral intensity measured at a particular wavelength over time. Scroll bars are provided if plots are out of view.

Each row in the sample measurements table below the plots shows the measurement results for one sample at a specified wavelength. The table contains information specified on the Reportable Data tab in Reports, plus the T_m value if **Calculate Tm values automatically at end of data acquisition** is selected on the **Settings > Tm Calculations** tab.



#	Sample ID	Date and Time	Tm Calculation Method	Temperature1	Temperature2	Temperature3	Tm Result
1	260nm, Sample1	10/4/2013 3:30:43 PM	Horizontal Intercept	72	No Value	92	83.434

To extend the collection time for the current sample measurement (without interrupting the data collection), click **Extend Time** in the navigation pane and enter the amount of time to extend the measurement. If you are collecting data in multiple stages, only the current stage is affected.

Right-click the **Data tab** to access features for changing the scale or adding annotation. See Data Display for more information.

To measure a sample again, right-click its row in the sample measurements table and choose **Remeasure** (if available). (If Remeasure is not available, you can choose **Options > Preferences** and clear **Prevent removal of data** to enable it.) After the remeasurement, the previous information for the sample is either crossed out or removed from the table, depending on the status of **Prevent removal of data** on the Preferences tab in Options.

To copy the data, right-click the plot and choose **Copy to Clipboard**.

7. To calculate or modify the DNA melting temperature (T_m) calculations on the data in a plot, **double-click the plot** or select **Analyze** (menu) > Tm Calculations.

Related Topics

Overview

DNA Melting Point Calculations

Application Settings

Unique Screen Features

Modifying T_m Calculations

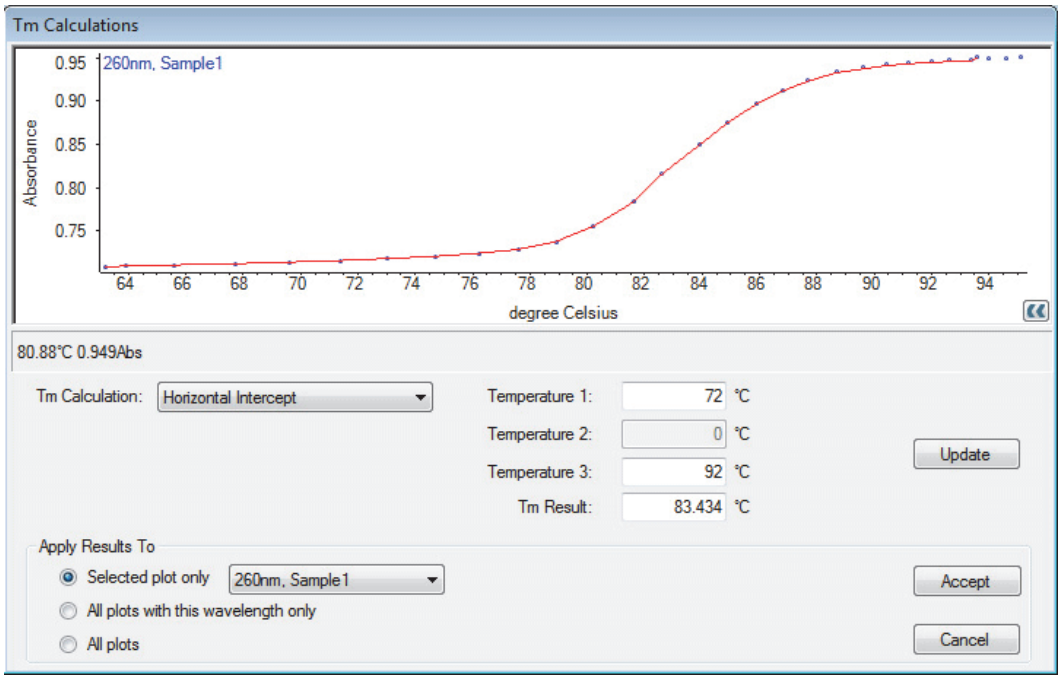
Data Display

Modifying T_m Calculations

DNA melting measurements must define how the DNA melting point will be measured. This is done on the Tm Calculations tab in Settings.

If **Calculate T_m values automatically at end of data acquisition** is selected on the T_m Calculations tab, after data collection completes, the calculated T_m values will appear in the sample measurements table along with the other sample data. To perform T_m calculations or modify existing T_m calculations on the data in a plot, double-click the plot or select **Analyze** (menu) > **T_m Calculations**. A window displays the plot and a table containing the current T_m calculation values (based on the T_m Calculations tab in Settings).

These operations are available from the T_m Calculations window:



- If desired, change the **Tm Calculation** setting to best fit the shape of the DNA melting point curve. If you select a different calculation method, add or change the required temperature values as needed. For more information, see DNA Melting Point Calculations.
- Click **Update** to recalculate Tm values based on the selected calculation method.
- Specify how to apply the calculations and then click **Accept**.

This option...	Applies the calculations to...
Selected plot only	The selected data only.
All plots with this wavelength only	All plots for the current wavelength.
All plots	All plots.

After you click Accept, the window closes and the sample measurements table is updated with the new Tm values.

Related Topics

Overview

DNA Melting Point Calculations

Application Settings

Unique Screen Features

Making DNA Melting Measurements

Protein A280

Contents

Overview

The Protein A280 method is used to quantify proteins on the basis of their absorbance at 280 nm.

Tyrosine, tryptophan, phenylalanine and to a small extent lysine and Cys-Cys disulphide bonds have an absorbance peak around 280 nm. This method does not require generation of a standard curve and is ready for protein sample quantitation at software startup. Colorimetric assays such as Pierce BCA, Pierce 660 nm, Bradford and Lowry are more commonly used for uncharacterized protein solutions and cell lysates.

The Protein A280 application displays the UV spectrum, measures the protein's absorbance at 280 nm (A280), and calculates the concentration (mg/ml).

Related Topics

Application Settings

Data Display

Unique Screen Features

Making Protein A280 Measurements

Application Settings

To set data acquisition parameters for a Protein A280 workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Protein A280
Measurement Tab for Protein A280
Instrument Tab for Protein A280
Accessories Tab for Protein A280
Samples Tab for Protein A280

Related Topics

Overview

Data Display

Unique Screen Features

Making Protein A280 Measurements

Type Tab for Protein A280

Click **Settings** in Protein A280 to display the Type tab in the right pane.

Feature	Description
Name	Application name.
Description (optional)	Description of the template.

Feature	Description
Protein A280 Type	Type of analysis to perform. **1 Abs = 1 mg / mL** is a general reference setting based on a 0.1% (1 mg/ml) protein solution. It produces an absorbance of 1.0 A at 280 nm (where the pathlength is 10 mm, or 1 cm). **BSA** is a Bovine Serum Albumin reference. Protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/ml) BSA solution. **IgG** is an IgG reference. Protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution. For **Lysozyme**, protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (10 mg/ml) Lysozyme solution. **Other protein (ε & MW)** is the entered molar extinction coefficient (M^{-1} cm^{-1}) and molecular weight (MW) in kilodaltons (kDa) for the respective protein reference. The maximum values are 99999000 for ε and 9999000 for MW. **Other protein (ε 1%)** is the mass extinction coefficient (l g^{-1} cm^{-1}) for a 10 mg/ml (1%) solution of the respective reference protein. Enter an ε/1000 value.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Protein A280

Instrument Tab for Protein A280

Accessories Tab for Protein A280

Samples Tab for Protein A280

Measurement Tab for Protein A280

	Click **Settings** in Protein A280 to display the Measurement tab in the right pane.

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Protein A280

Instrument Tab for Protein A280

Accessories Tab for Protein A280

Samples Tab for Protein A280

Instrument Tab for Protein A280

Click **Settings** in Protein A280 to display the Instrument tab in the right pane.

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Protein A280

Measurement Tab for Protein A280

Accessories Tab for Protein A280

Samples Tab for Protein A280

Accessories Tab for Protein A280

Click **Settings** in Protein A280 to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Protein A280

Measurement Tab for Protein A280

Instrument Tab for Protein A280

Samples Tab for Protein A280

Samples Tab for Protein A280

Click **Settings** in Protein A280 to display the Samples tab in the right pane.

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.

Feature	Description
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Protein A280

Measurement Tab for Protein A280

Instrument Tab for Protein A280

Accessories Tab for Protein A280

Unique Screen Features

The right pane in Measure Protein A280 may include:

Feature	Description
Type	Type of analysis to perform.
ε/1000 and Molecular Weight (kDa)	ε/1000 and molecular weight for the Other protein (ε & MW) sample type.
Ext. Coeff, ε 1% (L/g-cm)	Extinction coefficient and ε 1% for the Other protein (ε 1%) sample type.
Conc.	Concentration based on absorbance at 280 nm, after correction is applied, and the selected extinction coefficient.
A280	Absorbance at 280 nm for the protein sample being measured.
260/280	Ratio of absorbance at 260 nm and 280 nm.

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.
4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).
7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.

9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Application Settings

Data Display

Making Protein A280 Measurements

Making Protein A280 Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

❖ To make a Protein A280 measurement

1. Click **Measure Protein A280** in Protein A280.

Note To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. Click **Measure**.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank, generally the same buffer used to prepare or dissolve the sample. The buffer should be the same pH and of a similar ionic strength as the sample solution.

4. When samples information appears, modify it if desired.

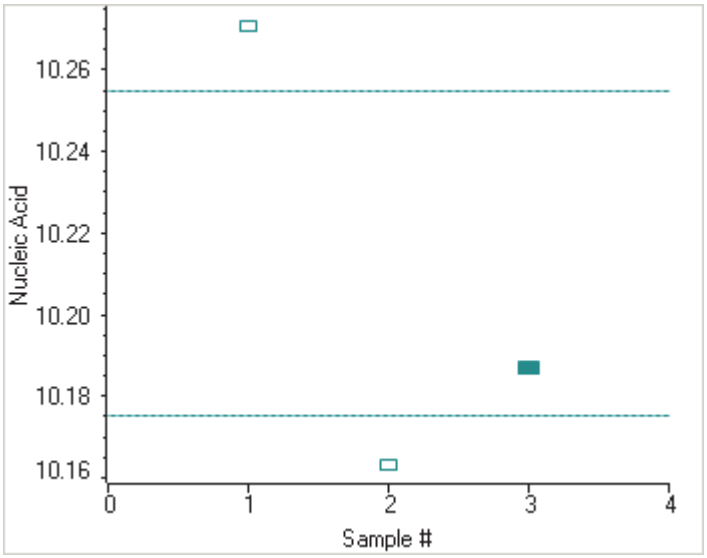
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

5. If only one sample will be measured, insert it.
6. Click **Continue**.
7. Follow any instructions that appear, such as to install a specified sample.

The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Related Topics

Overview

Application Settings

Data Display

Unique Screen Features

Making Protein A280 Measurements

Proteins and Labels

Contents

Overview

The Proteins & Labels application determines protein concentration using the protein's intrinsic absorption at 280 nm as well as the concentration of a fluorescent dye modification (protein array conjugates). It can also measure the purity of metalloproteins (such as hemoglobin) using wavelength ratios.

Related Topics

Dye/Chromophore Editor

Application Settings

Data Display

Unique Screen Features

Making Proteins and Labels Measurements

Dye/Chromophore Editor

Use the Dye Chromophore Editor to select predefined dyes, modify existing dyes for protocols, or enter new dyes.

To enter a new dye, type its information in the appropriate cells of the bottom row of the table and select a unit. Refer to the dye manufacturer for appropriate correction factors. The 260 nm corrections will be used for nucleic acid sample concentration calculations. Entered information is saved automatically.

To delete a user-defined dye, right-click its row and choose **Delete Row**. Predefined dyes, identified by the lock icon, cannot be edited or deleted.

Related Topics

Overview

Application Settings

Data Display

Unique Screen Features

Making Proteins and Labels Measurements

Application Settings

To set data acquisition parameters for a Proteins and Labels workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Proteins and Labels
Measurement Tab for Proteins and Labels
Instrument Tab for Proteins and Labels
Accessories Tab for Proteins and Labels
Samples Tab for Proteins and Labels

Related Topics

Overview

Dye/Chromophore Editor

Data Display

Unique Screen Features

Making Proteins and Labels Measurements

Type Tab for Proteins and Labels

Click **Settings** in Proteins and Labels to display the Type tab in the right pane.

Feature	Description
Name	Application name.
Description (optional)	Description of the template.

Feature	Description
Type	Type of analysis to perform. **1 Abs = 1 mg / mL** is a general reference setting based on a 0.1% (1 mg/ml) protein solution. It produces an absorbance of 1.0 A at 280 nm (where the pathlength is 10 mm, or 1 cm). **BSA** is a Bovine Serum Albumin reference. Protein concentrations are calculated using the mass extinction coefficient of 6.7 at 280 nm for a 1% (10 mg/ml) BSA solution. **IgG** is an IgG reference. Protein concentrations are calculated using the mass extinction coefficient of 13.7 at 280 nm for a 1% (10 mg/ml) IgG solution. For **Lysozyme**, protein concentrations are calculated using the mass extinction coefficient of 26.4 at 280 nm for a 1% (10 mg/ml) Lysozyme solution. **Other protein (ε & MW)** is the entered molar extinction coefficient (M^{-1} cm^{-1}) and molecular weight (MW) in kilodaltons (kDa) for the respective protein reference. The maximum values are 99999 X 1000 for ε and 9999 X 1000 for MW **Other protein (ε 1%)** is the mass extinction coefficient ($l\ g^{-1}$ cm^{-1}) for a 10 mg/ml (1%) solution of the respective reference protein.
Dye 1 and Dye 2	Fluorescent dyes whose absorbance will be used in concentration correction calculations.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Proteins and Labels

Instrument Tab for Proteins and Labels

Accessories Tab for Proteins and Labels

Samples Tab for Proteins and Labels

Measurement Tab for Proteins and Labels

	Click **Settings** in Proteins and Labels to display the Measurement tab in the right pane.

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Proteins and Labels

Instrument Tab for Proteins and Labels

Accessories Tab for Proteins and Labels

Samples Tab for Proteins and Labels

Instrument Tab for Proteins and Labels

Click **Settings** in Proteins and Labels to display the Instrument tab in the right pane.

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Proteins and Labels

Measurement Tab for Proteins and Labels

Accessories Tab for Proteins and Labels

Samples Tab for Proteins and Labels

Accessories Tab for Proteins and Labels

Click **Settings** in Proteins and Labels to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Proteins and Labels

Measurement Tab for Proteins and Labels

Instrument Tab for Proteins and Labels

Samples Tab for Proteins and Labels

Samples Tab for Proteins and Labels

Click **Settings** in Proteins and Labels to display the Samples tab in the right pane.

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.

Feature	Description
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Proteins and Labels

Measurement Tab for Proteins and Labels

Instrument Tab for Proteins and Labels

Accessories Tab for Proteins and Labels

Unique Screen Features

The right pane in Measure Protein & Labels includes:

Feature	Description
Type	Type of analysis to perform.
ε/1000 and Molecular Weight (kDa)	ε/1000 and molecular weight for the Other protein (ε & MW) sample type.
Ext. Coeff, ε 1% (L/g-cm)	Extinction coefficient and ε 1% for the Other protein (ε 1%) sample type.

Feature	Description
Conc.	Concentration based on absorbance at 280 nm, after correction is applied, and the selected extinction coefficient.
Dye(s) Conc.	Concentration of the user-selected dye(s). The software automatically subtracts the value of a sloping baseline from 400 to 750 nm from the absorbance at the Dye wavelength. Only the corrected absorbance of the dye peak(s) and dye concentration(s) are reported.

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.

4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).
7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.
9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Dye/Chromophore Editor

Making Proteins and Labels Measurements

While performing this procedure, click here for information about features to the right of the spectral display.

❖ To make a Proteins and Labels measurement

1. Click **Measure Proteins & Labels** in Proteins and Labels.

Note To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. Click **Measure**.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank, generally the same buffer used to prepare or dissolve the sample. This solution should be the same pH and of a similar ionic strength as the sample solution.

4. When samples information appears, modify it if desired.

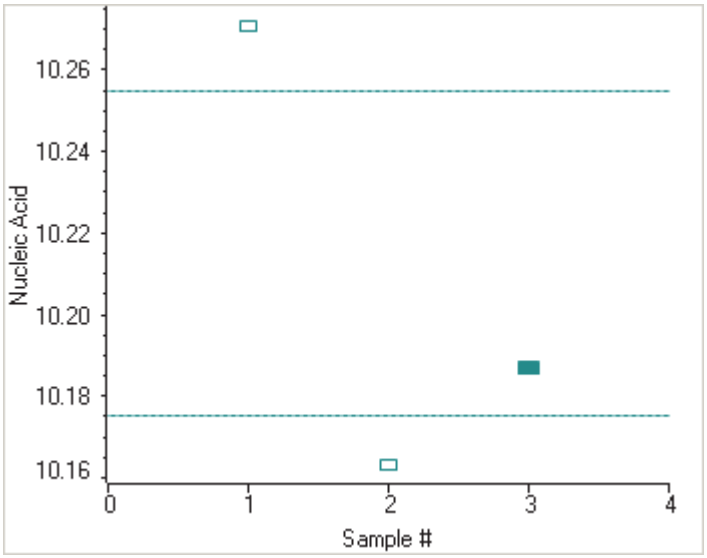
More:

If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

5. If only one sample will be measured, insert it.
6. Click **Continue**.
7. Follow any instructions that appear, such as to install a specified sample.

The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To copy this plot, right-click it and choose **Copy to Clipboard**.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the sample selected in the table. (Right-click the data to access commands for customizing the display, including adding annotation. See Data Display for more information.)

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

The A280 value in the result table is the value using the absorbance at 750 nm as the baseline. The A280 value used to calculate the protein concentration takes into account the appropriate dye correction factors and any absorbance correction due to the selected analysis correction nm. Therefore, the displayed A280 value is not the value used to calculate the sample concentration.

To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

Related Topics

Overview

Dye/Chromophore Editor

Application Settings

Data Display

Unique Screen Features

Data Display

Pierce BCA

Contents

Overview

The BCA Protein Assay, which was patented by Pierce Biotechnology, part of Thermo Fisher Scientific, remains a cornerstone for accurately measuring protein concentration in biological samples. The method uses bicinchoninic acid (BCA) as the detection reagent for Cu^{+}, which is formed when Cu^{2+} is reduced by protein in an alkaline environment. A purple-colored reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu^{+}). The resulting Cu-BCA chelate formed in the presence of protein is measured at 562 nm. While this assay is linear over a wide concentration range, the calibration curves are best represented as second order. Preformulated reagents of BCA and $CuSO_4$ are available in kit form from us.

BCA Kits and Protocols

Commercial BCA Protein kit manufacturers typically outline procedures for two different protein concentration ranges:

- A regular assay uses a 20:1 reagent/sample volume ratio.
- A mini assay uses a 1:1 reagent/sample volume ratio. To prepare sufficient volume of these 1:1 mixtures for cuvette measurements, use a sample volume of at least 3 ml and 3 ml of BCA reagent.

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

Note If running the assay at 60 °C, doubling the volumes may afford greater insurance against skewed results from evaporation/condensation within the sealed reaction tube.

Protein standards (BSA) for generating a standard curve may also be provided by the manufacturer for the BCA assay.

Working With Standard Curves

A standard curve is required for colorimetric protein analysis. Here are tips on creating a standard curve:

- A standard curve can be generated using two or more standards.
- The multipoint curve capability allows multiple replicates for each standard. Standards can be run in any order; however, best practice dictates that the standards be measured from the lowest concentration to the highest.
- Determine whether or not to use a standard when analyzing samples against the standard curve.
- Establishing a new standard curve requires creating a new workbook.
- If a previously saved workbook is used, all concentration calculations for newly measured samples will be based on the standard absorbance values saved in the workbook.

Note Each workbook will archive only one standard curve.

Related Topics

Application Settings

Unique Screen Features

Data Display

Making Pierce BCA Measurements

Application Settings

To set data acquisition parameters for a Pierce BCA workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Pierce BCA
Measurement Tab for Pierce BCA
Standards Tab for Pierce BCA
Instrument Tab for Pierce BCA
Accessories Tab for Pierce BCA
Samples Tab for Pierce BCA

Related Topics

Overview

Unique Screen Features

Data Display

Making Pierce BCA Measurements

Type Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Type tab in the right pane.

Feature	Description
Name	Application name.
Description (optional)	Description of the template.
Standard curve	Type of quantitative analysis used.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Pierce BCA

Standards Tab for Pierce BCA

Instrument Tab for Pierce BCA

Accessories Tab for Pierce BCA

Samples Tab for Pierce BCA

Measurement Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Measurement tab in the right pane.

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Pierce BCA

Standards Tab for Pierce BCA

Instrument Tab for Pierce BCA

Accessories Tab for Pierce BCA

Samples Tab for Pierce BCA

Standards Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Standards tab in the right pane.

Feature	Description
Curve fit type	Type of equation used to create the standard curve from standard concentration values.
Standard averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.
Use correction factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Contains concentration and other information about the standards. To add information from a .csv (comma separated values) file or .tsv (tab separated values) file, click **Import Standards**. To save the information in a .csv file or .tsv file, click **Export Standards**.

Related Topics

Working With Standard Curves

Type Tab for Pierce BCA

Measurement Tab for Pierce BCA

Instrument Tab for Pierce BCA

Accessories Tab for Pierce BCA

Samples Tab for Pierce BCA

Instrument Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Instrument tab in the right pane.

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Pierce BCA

Measurement Tab for Pierce BCA

Standards Tab for Pierce BCA

Accessories Tab for Pierce BCA

Samples Tab for Pierce BCA

Accessories Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Pierce BCA

Measurement Tab for Pierce BCA

Standards Tab for Pierce BCA

Instrument Tab for Pierce BCA

Samples Tab for Pierce BCA

Samples Tab for Pierce BCA

Click **Settings** in Pierce BCA to display the Samples tab in the right pane.

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.

Feature	Description
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use weight/volume correction	If available for the current unit, corrects sample concentrations using the entered target weight and volume: corrected concentration = measured concentration * (actual weight / target weight) * (target volume / actual volume)
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Pierce BCA

Measurement Tab for Pierce BCA

Standards Tab for Pierce BCA

Instrument Tab for Pierce BCA

Accessories Tab for Pierce BCA

Unique Screen Features

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.
4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).

7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.
9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Application Settings

Data Display

Making Pierce BCA Measurements

Making Pierce BCA Measurements

Sample and Standards Preparation

- See the manufacturers' guidelines and recommendations for standard and sample preparation.
- Prepare both standards and unknowns in the same manner. Use a diluent of the same pH and ionic strength for all blanks, standards and unknown samples.

Note Standards diluted from a stock standard must cover the expected range of the unknown samples. Protein concentrations are not extrapolated beyond measured standard concentrations.

Procedure

❖ To make a Pierce BCA measurement

1. Click **Measure Pierce BCA** in Pierce BCA.

Note To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. If standards need to be acquired, click **Measure**. If standards have already been acquired, skip to step 7 to acquire sample data.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank. See the reagent directions for details.

4. When the standards information appears, modify it if desired.

   More:

   If Standard averaging on the Standards tab in Settings was set to Duplicate, "D" at the end of a standard name indicates the second measurement to be made of the standard. If Sample averaging was set to Triplicate, "D" and "T" at the end of standard names indicate the second and third measurements to be made, respectively.

   To enter previously saved standards information, use **Import Standards**.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each standard in the table.

To remove a standard, select it, right-click the table and choose **Clear Standard**. To remove all the standards, choose **Clear Table**.

To save standards information for later use, use **Export Standards**.

5. Click **Continue**.
6. Follow the instructions that appear, installing the specified standards.

   The Standard Curve(s) tab displays the resulting standard curve (or curves). Specify the standards to use for the curve (or curves) by selecting **Yes** or **No** in the **Use** column in the table.

   More:

   If a standard curve is not calibrated...

   - Try selecting a different curve fit type.
   - Try remeasuring a standard using the correct standard material: select the listed standard, right-click the table and choose **Remeasure**.
   - Try changing the setting of **Minimum r^2** on the Standards tab and acquire the standards again in a new workbook.

   This is only an indicator that the required minimum number of points have been established for the selected curve fit type. It does not validate the integrity of the curve. For example, additional standards may be required to cover the expected assay concentration range.

   The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

   If Standard averaging was set to Duplicate, “D” at the end of a standard name indicates the second measurement. If Standard averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

   To permanently remove an acquired standard from the analysis, right-click it and choose **Remove**. Its information is crossed out but not removed from the table.

   The Data tab displays the acquired data (a fixed data point or scan spectrum) for the standard selected in the table. To access commands for customizing the display, including adding annotation, right-click the data. See Data Display for more information.

7. Click **Measure**.

8. Follow the instructions that appear.
9. When samples information appears, modify it if desired.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each sample in the table.

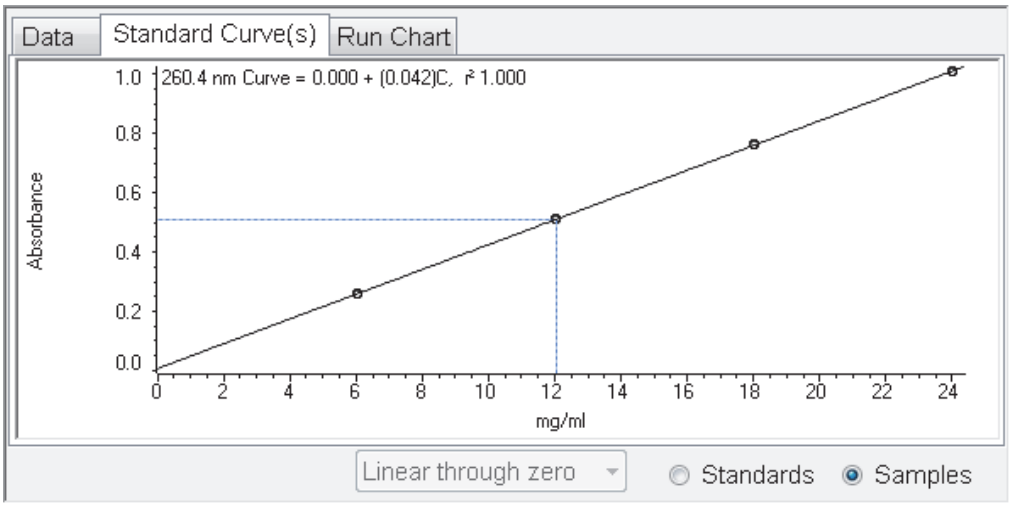
   More:

   If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

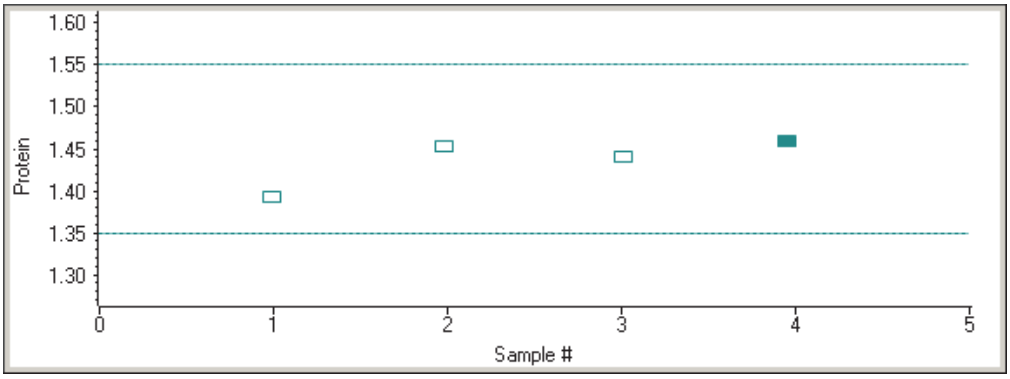
   To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.
10. If only one sample will be measured, install it.
11. Click **Continue**.
12. Follow any instructions that appear, such as to install a specified sample.

    The sample results table contains the columns of information specified on the Configuration tab in Reports. If Sample averaging was set to Duplicate, “D” at the end of a sample name indicates the second measurement. If Sample averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

    The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis:



    The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

To copy the data on the Standard Curve(s) tab or Run Chart tab, right-click the plot and choose **Copy to Clipboard**.

Related Topics

Overview

Application Settings

Data Display

Unique Screen Features

Working With Standard Curves

Protein Bradford

Contents

Overview

Protein Bradford	The Protein Bradford assay measures absorbance at 595 nm; it determines concentration for either standard or micro sample concentrations.

This assay is commonly used for determining protein concentration. It is often used for more dilute protein solutions where lower detection sensitivity is needed and/or in the presence of components that also have significant UV (280 nm) absorbance. Like the other colorimetric assays, the Bradford assay requires generating a standard curve before measuring sample proteins.

The Bradford procedure uses the protein-induced absorbance shift of Coomassie Blue dye to 595 nm to measure protein concentration. The bound protein-dye complex is measured at 595 nm and normalized at 750 nm. A single stabilized reagent mixture containing Coomassie Blue dye, alcohol, and surfactant in kit form is available from numerous manufacturers.

Bradford Reagent Kits and Protocols

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns), ensuring they are subjected to the same timing and temperature throughout the assay.

Protein standards (BSA) for generating a standard curve may also be provided by the Bradford assay manufacturer.

Working With Standard Curves

A standard curve is required for colorimetric protein analysis. Here are tips on creating a standard curve:

- A standard curve can be generated using two or more standards.
- The multipoint curve capability allows multiple replicates for each standard. Standards can be run in any order; however, best practice dictates that the standards be measured from the lowest concentration to the highest.
- Determine whether or not to use a standard when analyzing samples against the standard curve.
- Establishing a new standard curve requires creating a new workbook.
- If a previously saved workbook is used, all concentration calculations for newly measured samples will be based on the standard absorbance values saved in the workbook.

> **Note** Each workbook will archive only one standard curve.

Related Topics

Application Settings

Data Display

Unique Screen Features

Making Protein Bradford Assay Measurements

Application Settings

To set data acquisition parameters for a Protein Bradford workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Protein Bradford
Measurement Tab for Protein Bradford
Standards Tab for Protein Bradford
Instrument Tab for Protein Bradford
Accessories Tab for Protein Bradford
Samples Tab for Protein Bradford

Related Topics

Overview

Data Display

Unique Screen Features

Making Protein Bradford Assay Measurements

Type Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Type tab in the right pane.

Feature	Description
Name	Application name.
Description (optional)	Description of the template.
Standard curve	Type of quantitative analysis used.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Protein Bradford

Standards Tab for Protein Bradford

Instrument Tab for Protein Bradford

Accessories Tab for Protein Bradford

Samples Tab for Protein Bradford

Measurement Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Measurement tab in the right pane.

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Protein Bradford

Standards Tab for Protein Bradford

Instrument Tab for Protein Bradford

Accessories Tab for Protein Bradford

Samples Tab for Protein Bradford

Standards Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Standards tab in the right pane.

Feature	Description
Curve fit type	Type of equation used to create the standard curve from standard concentration values.
Standard averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.
Use correction factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Contains concentration and other information about the standards. To add information from a .csv (comma separated values) file or .tsv (tab separated values) file, click **Import Standards**. To save the information in a .csv file or .tsv file, click **Export Standards**.

Related Topics

Working With Standard Curves

Type Tab for Protein Bradford

Measurement Tab for Protein Bradford

Instrument Tab for Protein Bradford

Accessories Tab for Protein Bradford

Samples Tab for Protein Bradford

Instrument Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Instrument tab in the right pane.

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Protein Bradford

Measurement Tab for Protein Bradford

Standards Tab for Protein Bradford

Accessories Tab for Protein Bradford

Samples Tab for Protein Bradford

Accessories Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Protein Bradford

Measurement Tab for Protein Bradford

Standards Tab for Protein Bradford

Instrument Tab for Protein Bradford

Samples Tab for Protein Bradford

Samples Tab for Protein Bradford

Click **Settings** in Protein Bradford to display the Samples tab in the right pane.

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.

Feature	Description
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use weight/volume correction	If available for the current unit, corrects sample concentrations using the entered target weight and volume: corrected concentration = measured concentration * (actual weight / target weight) * (target volume / actual volume)
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Protein Bradford

Measurement Tab for Protein Bradford

Standards Tab for Protein Bradford

Instrument Tab for Protein Bradford

Accessories Tab for Protein Bradford

Unique Screen Features

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.
4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).

7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.
9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Application Settings

Data Display

Making Protein Bradford Assay Measurements

Making Protein Bradford Assay Measurements

See Sample and Standards Preparation for more information.

❖ To make Bradford assay measurements

1. Click **Measure Protein Bradford** in Protein Bradford.

Note To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. If standards need to be acquired, click **Measure**. If standards have already been acquired, skip to step 7 to acquire sample data.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank. See the reagent directions for details.

4. When the standards information appears, modify it if desired.

   More:

   If Standard averaging on the Standards tab in Settings was set to Duplicate, “D” at the end of a standard name indicates the second measurement to be made of the standard. If Sample averaging was set to Triplicate, “D” and “T” at the end of standard names indicate the second and third measurements to be made, respectively.

   To enter previously saved standards information, use **Import Standards**.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each standard in the table.

   To remove a standard, select it, right-click the table and choose **Clear Standard**. To remove all the standards, choose **Clear Table**.

   To save standards information for later use, use **Export Standards**.

5. Click **Continue**.

6. Follow the instructions that appear, installing the specified standards.

   The Standard Curve(s) tab displays the resulting standard curve (or curves). Specify the standards to use for the curve (or curves) by selecting **Yes** or **No** in the **Use** column in the table.

   More:

Method Uncalibrated

If a standard curve is not calibrated...

- Try selecting a different curve fit type.
- Try remeasuring a standard using the correct standard material: select the listed standard, right-click the table and choose **Remeasure**.
- Try changing the setting of **Minimum r^2** on the Standards tab and acquire the standards again in a new workbook.

Method Calibrated

This is only an indicator that the required minimum number of points have been established for the selected curve fit type. It does not validate the integrity of the curve. For example, additional standards may be required to cover the expected assay concentration range.

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

If Standard averaging was set to Duplicate, “D” at the end of a standard name indicates the second measurement. If Standard averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

To permanently remove an acquired standard from the analysis, right-click it and choose **Remove**. Its information is crossed out but not removed from the table.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the standard selected in the table. To access commands for customizing the display, including adding annotation, right-click the data. See Data Display for more information.

7. Click **Measure**.

8. Follow the instructions that appear.
9. When samples information appears, modify it if desired.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each sample in the table.

   More:

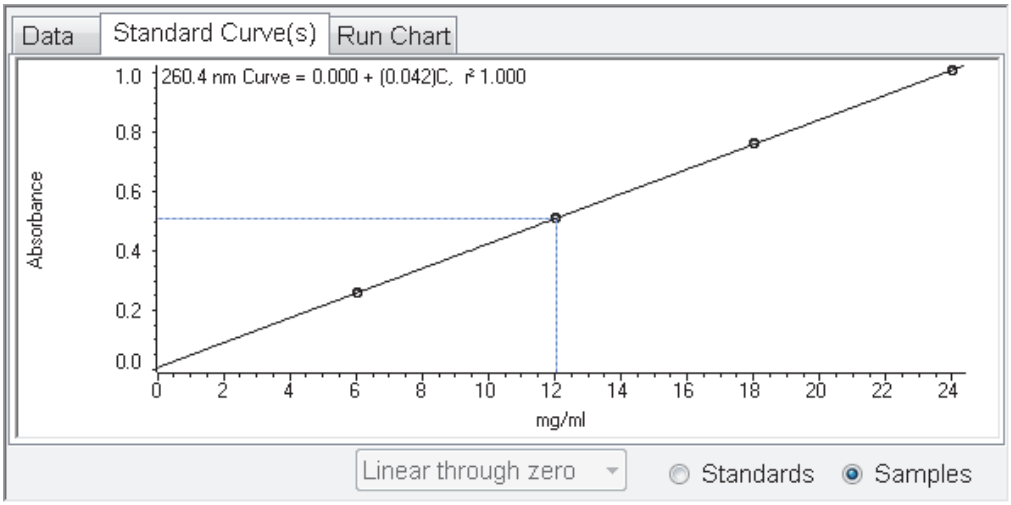
   If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

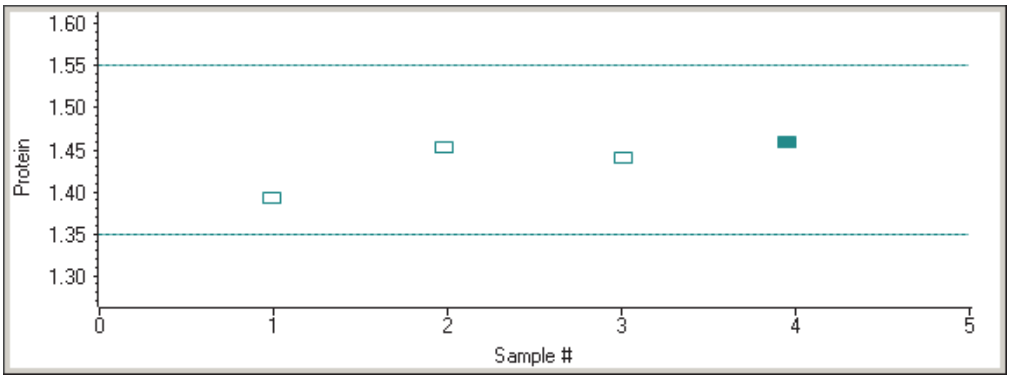
10. If only one sample will be measured, install it.
11. Click **Continue**.
12. Follow any instructions that appear, such as to install a specified sample.

The sample results table contains the columns of information specified on the Configuration tab in Reports. If Sample averaging was set to Duplicate, “D” at the end of a sample name indicates the second measurement. If Sample averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis:



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

To copy the data on the Standard Curve(s) tab or Run Chart tab, right-click the plot and choose **Copy to Clipboard**.

Related Topics

Overview

Application Settings

Data Display

Unique Screen Features

Working With Standard Curves

Pierce Modified Lowry

Contents

Overview

The Pierce Modified Lowry assay measures absorbance at 750 nm to determine concentration.

This assay is an alternative method for determining protein concentration based on the widely used and cited Lowry procedure for protein quantitation. Like the other colorimetric assays, the Lowry assay requires generating a standard curve before measuring sample proteins.

The Pierce Modified Lowry procedure involves reaction of protein with cupric sulfate in alkaline solution, resulting in formation of tetradentate copper-protein complexes. The Folin-Ciocalteu Reagent is effectively reduced in proportion to the chelated copper-complexes, resulting in a water-soluble blue product that is measured at 650 nm and normalized at 405 nm. Preformulated reagents, used in the assay, are available in kit form from numerous manufacturers.

Modified Lowry Kits and Protocols

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns). Ensure each is subjected to the same timing and temperature throughout the assay.

Protein standards (BSA) for generating a standard curve may also be provided by the manufacturer for the Lowry assay.

Working With Standard Curves

A standard curve is required for colorimetric protein analysis. Here are tips on creating a standard curve:

- A standard curve can be generated using two or more standards.
- The multipoint curve capability allows multiple replicates for each standard. Standards can be run in any order; however, best practice dictates that the standards be measured from the lowest concentration to the highest.
- Determine whether or not to use a standard when analyzing samples against the standard curve.
- Establishing a new standard curve requires creating a new workbook.
- If a previously saved workbook is used, all concentration calculations for newly measured samples will be based on the standard absorbance values saved in the workbook.

Note Each workbook will archive only one standard curve.

Related Topics

Application Settings

Data Display

Unique Screen Features

Making Pierce Modified Lowry Assay Measurements

Application Settings

To set data acquisition parameters for a Pierce Modified Lowry workbook, click **Settings.**

The follow tabs of settings are available:
Type Tab for Pierce Modified Lowry
Measurement Tab for Pierce Modified Lowry
Standards Tab for Pierce Modified Lowry
Instrument Tab for Pierce Modified Lowry
Accessories Tab for Pierce Modified Lowry
Samples Tab for Pierce Modified Lowry

Related Topics

Overview

Data Display

Unique Screen Features

Making Pierce Modified Lowry Assay Measurements

Type Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Type tab in the right pane.

Feature	Description
Name	Application name.
Description (optional)	Description of the template.
Standard curve	Type of quantitative analysis used.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Pierce Modified Lowry

Standards Tab for Pierce Modified Lowry

Instrument Tab for Pierce Modified Lowry

Accessories Tab for Pierce Modified Lowry

Samples Tab for Pierce Modified Lowry

Measurement Tab for Pierce Modified Lowry

Click **Settings** in Protein Lowry to display the Measurement tab in the right pane.

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Pierce Modified Lowry

Standards Tab for Pierce Modified Lowry

Instrument Tab for Pierce Modified Lowry

Accessories Tab for Pierce Modified Lowry

Samples Tab for Pierce Modified Lowry

Standards Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Standards tab in the right pane.

Feature	Description
Curve fit type	Type of equation used to create the standard curve from standard concentration values.
Standard averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.
Use correction factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Contains concentration and other information about the standards. To add information from a .csv (comma separated values) file or .tsv (tab separated values) file, click **Import Standards**. To save the information in a .csv file or .tsv file, click **Export Standards**.

Related Topics

Working With Standard Curves

Type Tab for Pierce Modified Lowry

Measurement Tab for Pierce Modified Lowry

Instrument Tab for Pierce Modified Lowry

Accessories Tab for Pierce Modified Lowry

Samples Tab for Pierce Modified Lowry

Instrument Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Instrument tab in the right pane.

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Pierce Modified Lowry

Measurement Tab for Pierce Modified Lowry

Standards Tab for Pierce Modified Lowry

Accessories Tab for Pierce Modified Lowry

Samples Tab for Pierce Modified Lowry

Accessories Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Pierce Modified Lowry

Measurement Tab for Pierce Modified Lowry

Standards Tab for Pierce Modified Lowry

Instrument Tab for Pierce Modified Lowry

Samples Tab for Pierce Modified Lowry

Samples Tab for Pierce Modified Lowry

Click **Settings** in Pierce Modified Lowry to display the Samples tab in the right pane.

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.

Feature	Description
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use weight/volume correction	If available for the current unit, corrects sample concentrations using the entered target weight and volume: corrected concentration = measured concentration * (actual weight / target weight) * (target volume / actual volume)
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Pierce Modified Lowry

Measurement Tab for Pierce Modified Lowry

Standards Tab for Pierce Modified Lowry

Instrument Tab for Pierce Modified Lowry

Accessories Tab for Pierce Modified Lowry

Unique Screen Features

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.
4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).

7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.
9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Application Settings

Data Display

Making Pierce Modified Lowry Assay Measurements

Making Pierce Modified Lowry Assay Measurements

See Sample and Standards Preparation for more information.

❖ To make Pierce Modified Lowry Assay measurements

1. Click **Measure Pierce Modified Lowry** in Pierce Modified Lowry.

> **Note** To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. If standards need to be acquired, click **Measure**. If standards have already been acquired, skip to step 7 to acquire sample data.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank. See the reagent directions for details.

4. When the standards information appears, modify it if desired.

   More:

   If Standard averaging on the Standards tab in Settings was set to Duplicate, “D” at the end of a standard name indicates the second measurement to be made of the standard. If Sample averaging was set to Triplicate, “D” and “T” at the end of standard names indicate the second and third measurements to be made, respectively.

   To enter previously saved standards information, use **Import Standards**.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each standard in the table.

   To remove a standard, select it, right-click the table and choose **Clear Standard**. To remove all the standards, choose **Clear Table**.

   To save standards information for later use, use **Export Standards**.

5. Click **Continue**.

6. Follow the instructions that appear, installing the specified standards.

   The Standard Curve(s) tab displays the resulting standard curve (or curves). Specify the standards to use for the curve (or curves) by selecting **Yes** or **No** in the **Use** column in the table.

   More:

Method Uncalibrated

If a standard curve is not calibrated...

- Try selecting a different curve fit type.
- Try remeasuring a standard using the correct standard material: select the listed standard, right-click the table and choose **Remeasure**.
- Try changing the setting of **Minimum r^2** on the Standards tab and acquire the standards again in a new workbook.

Method Calibrated

This is only an indicator that the required minimum number of points have been established for the selected curve fit type. It does not validate the integrity of the curve. For example, additional standards may be required to cover the expected assay concentration range.

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

If Standard averaging was set to Duplicate, “D” at the end of a standard name indicates the second measurement. If Standard averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

To permanently remove an acquired standard from the analysis, right-click it and choose **Remove**. Its information is crossed out but not removed from the table.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the standard selected in the table. To access commands for customizing the display, including adding annotation, right-click the data. See Data Display for more information.

7. Click **Measure**.

8. Follow the instructions that appear.
9. When samples information appears, modify it if desired.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each sample in the table.

   More:

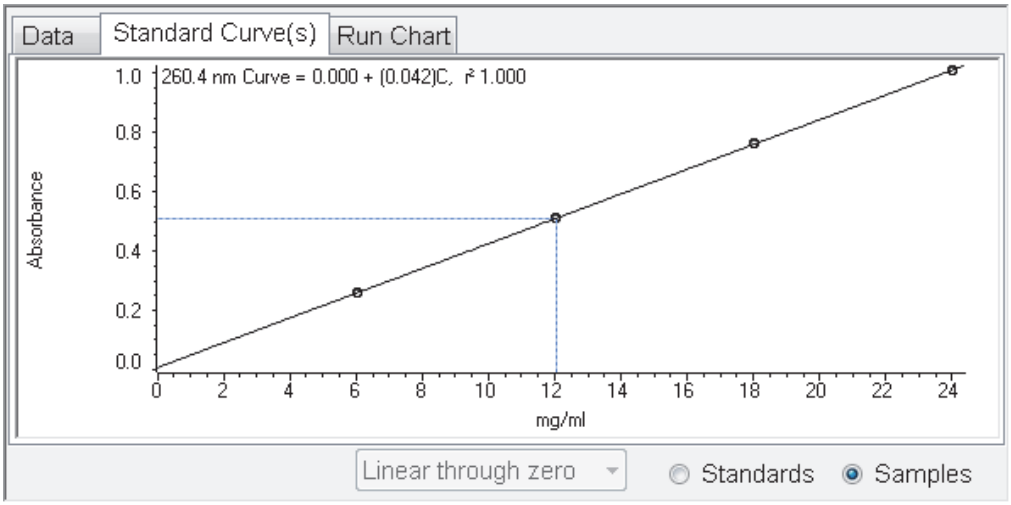
   If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

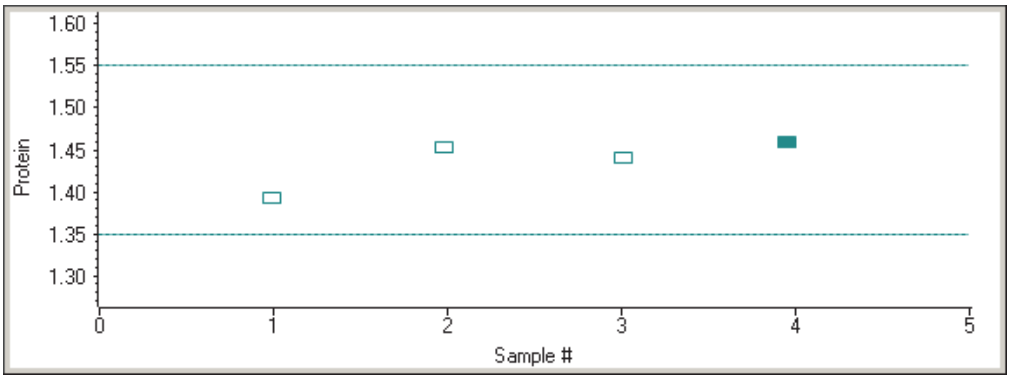
10. If only one sample will be measured, install it.
11. Click **Continue**.
12. Follow any instructions that appear, such as to install a specified sample.

The sample results table contains the columns of information specified on the Configuration tab in Reports. If Sample averaging was set to Duplicate, “D” at the end of a sample name indicates the second measurement. If Sample averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis:



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

To copy the data on the Standard Curve(s) tab or Run Chart tab, right-click the plot and choose **Copy to Clipboard**.

Related Topics

Overview

Application Settings

Data Display

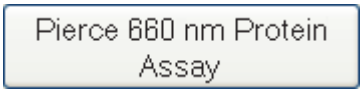
Unique Screen Features

Working With Standard Curves

Pierce 660 nm Protein Assay

Contents

Overview



The Thermo Scientific Pierce 660 nm Protein Assay reagent is a ready-to-use formulation that offers rapid, accurate and reproducible colorimetric detection of minute amounts of protein in solution. The reagent is ideal for measuring total protein concentration in samples containing reducing agents and/or detergents.

The proprietary dye-metal complex binds to protein in acidic conditions, causing a shift in the dye's absorption maximum, which is measured at 660 nm. The complex is reddish-brown and turns green upon protein binding. This color change is produced by deprotonation of the dye at low pH facilitated by interactions with positively charged amino acid groups in the protein. Consequently, the complex interacts primarily with basic residues in the protein, such as histidine, arginine and lysine, and to a lesser extent tyrosine, tryptophan and phenylalanine.

Pierce 660 nm Reagent and Protocols

Follow the assay kit manufacturer's recommendations for all standards and samples (unknowns), ensuring they are subjected to the same timing and temperature throughout the assay.

Protein standards (BSA) for generating a standard curve may also be provided by the Pierce 660 nm assay manufacturer.

Working With Standard Curves

A standard curve is required for colorimetric protein analysis. Here are tips on creating a standard curve:

- A standard curve can be generated using two or more standards.
- The multipoint curve capability allows multiple replicates for each standard. Standards can be run in any order; however, best practice dictates that the standards be measured from the lowest concentration to the highest.
- Determine whether or not to use a standard when analyzing samples against the standard curve.
- Establishing a new standard curve requires creating a new workbook.
- If a previously saved workbook is used, all concentration calculations for newly measured samples will be based on the standard absorbance values saved in the workbook.

Note Each workbook will archive only one standard curve.

Related Topics

Application Settings

Data Display

Unique Screen Features

Making Pierce 660 nm Protein Assay Measurements

Application Settings

To set data acquisition parameters for a Pierce 660 nm Protein Assay workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Pierce 660 nm Protein Assay
Measurement Tab for Pierce 660 nm Protein Assay
Standards Tab for Pierce 660 nm Protein Assay
Instrument Tab for Pierce 660 nm Protein Assay
Accessories Tab for Pierce 660 nm Protein Assay
Samples Tab for Pierce 660 nm Protein Assay

Related Topics

Overview

Data Display

Unique Screen Features

Making Pierce 660 nm Protein Assay Measurements

Type Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Type tab in the right pane.

Feature	Description
Name	Application name.
Description (optional)	Description of the template.
Standard curve	Type of quantitative analysis used.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Pierce 660 nm Protein Assay

Standards Tab for Pierce 660 nm Protein Assay

Instrument Tab for Pierce 660 nm Protein Assay

Accessories Tab for Pierce 660 nm Protein Assay

Samples Tab for Pierce 660 nm Protein Assay

Measurement Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Measurement tab in the right pane.

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Pierce 660 nm Protein Assay

Standards Tab for Pierce 660 nm Protein Assay

Instrument Tab for Pierce 660 nm Protein Assay

Accessories Tab for Pierce 660 nm Protein Assay

Samples Tab for Pierce 660 nm Protein Assay

Standards Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Standards tab in the right pane.

Feature	Description
Curve fit type	Type of equation used to create the standard curve from standard concentration values.
Standard averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.
Use correction factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Contains concentration and other information about the standards. To add information from a .csv (comma separated values) file or .tsv (tab separated values) file, click **Import Standards**. To save the information in a .csv file or .tsv file, click **Export Standards**.

Related Topics

Working With Standard Curves

Type Tab for Pierce 660 nm Protein Assay

Measurement Tab for Pierce 660 nm Protein Assay

Instrument Tab for Pierce 660 nm Protein Assay

Accessories Tab for Pierce 660 nm Protein Assay

Samples Tab for Pierce 660 nm Protein Assay

Instrument Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Instrument tab in the right pane.

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Pierce 660 nm Protein Assay

Measurement Tab for Pierce 660 nm Protein Assay

Standards Tab for Pierce 660 nm Protein Assay

Accessories Tab for Pierce 660 nm Protein Assay

Samples Tab for Pierce 660 nm Protein Assay

Accessories Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Pierce 660 nm Protein Assay

Measurement Tab for Pierce 660 nm Protein Assay

Standards Tab for Pierce 660 nm Protein Assay

Instrument Tab for Pierce 660 nm Protein Assay

Samples Tab for Pierce 660 nm Protein Assay

Samples Tab for Pierce 660 nm Protein Assay

Click **Settings** in Pierce 660 nm Protein Assay to display the Samples tab in the right pane.

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.

Feature	Description
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use weight/volume correction	If available for the current unit, corrects sample concentrations using the entered target weight and volume: corrected concentration = measured concentration * (actual weight / target weight) * (target volume / actual volume)
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Pierce 660 nm Protein Assay

Measurement Tab for Pierce 660 nm Protein Assay

Standards Tab for Pierce 660 nm Protein Assay

Instrument Tab for Pierce 660 nm Protein Assay

Accessories Tab for Pierce 660 nm Protein Assay

Unique Screen Features

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.
4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).

7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.
9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Application Settings

Data Display

Making Pierce 660 nm Protein Assay Measurements

Making Pierce 660 nm Protein Assay Measurements

See Sample and Standards Preparation for more information.

❖ To make Pierce 660 nm Protein assay measurements

1. Click **Measure Pierce 660 nm Protein Assay** in Pierce 660 nm Protein Assay.

> **Note** To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. If standards need to be acquired, click **Measure**. If standards have already been acquired, skip to step 7 to acquire sample data.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank. See the reagent directions for details.

4. When the standards information appears, modify it if desired.

   More:

   If Standard averaging on the Standards tab in Settings was set to Duplicate, “D” at the end of a standard name indicates the second measurement to be made of the standard. If Sample averaging was set to Triplicate, “D” and “T” at the end of standard names indicate the second and third measurements to be made, respectively.

   To enter previously saved standards information, use **Import Standards**.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each standard in the table.

   To remove a standard, select it, right-click the table and choose **Clear Standard**. To remove all the standards, choose **Clear Table**.

   To save standards information for later use, use **Export Standards**.

5. Click **Continue**.

6. Follow the instructions that appear, installing the specified standards.

   The Standard Curve(s) tab displays the resulting standard curve (or curves). Specify the standards to use for the curve (or curves) by selecting **Yes** or **No** in the **Use** column in the table.

   More:

Method Uncalibrated

If a standard curve is not calibrated...

- Try selecting a different curve fit type.
- Try remeasuring a standard using the correct standard material: select the listed standard, right-click the table and choose **Remeasure**.
- Try changing the setting of **Minimum r^2** on the Standards tab and acquire the standards again in a new workbook.

Method Calibrated

This is only an indicator that the required minimum number of points have been established for the selected curve fit type. It does not validate the integrity of the curve. For example, additional standards may be required to cover the expected assay concentration range.

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

If Standard averaging was set to Duplicate, “D” at the end of a standard name indicates the second measurement. If Standard averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

To permanently remove an acquired standard from the analysis, right-click it and choose **Remove**. Its information is crossed out but not removed from the table.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the standard selected in the table. To access commands for customizing the display, including adding annotation, right-click the data. See Data Display for more information.

7. Click **Measure**.

8. Follow the instructions that appear.
9. When samples information appears, modify it if desired.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each sample in the table.

   More:

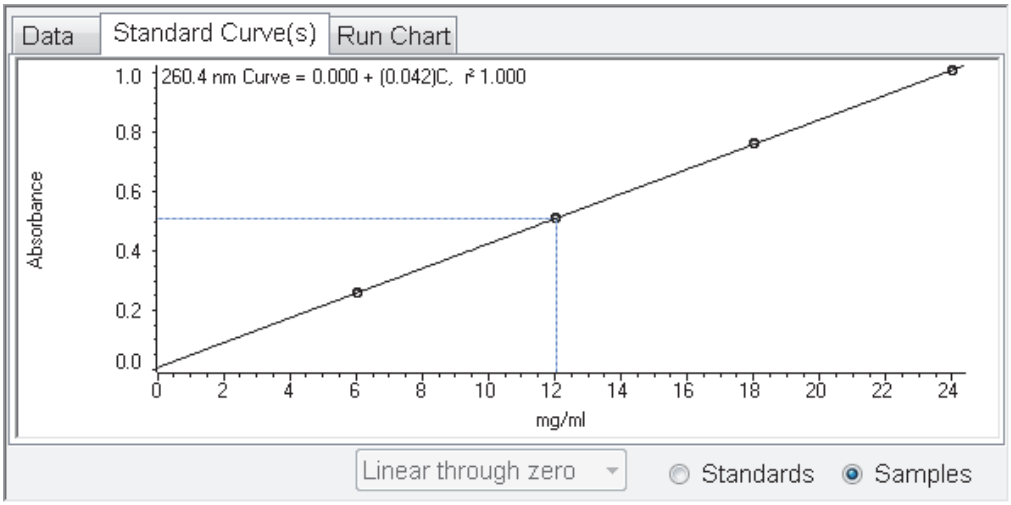
   If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

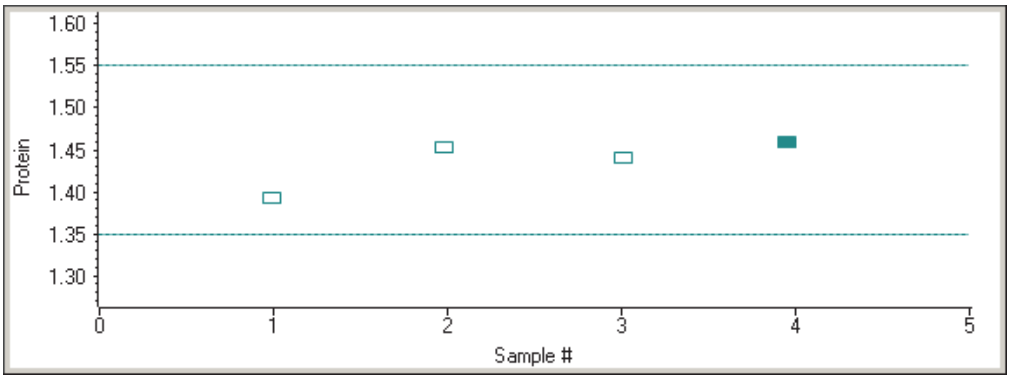
10. If only one sample will be measured, install it.
11. Click **Continue**.
12. Follow any instructions that appear, such as to install a specified sample.

The sample results table contains the columns of information specified on the Configuration tab in Reports. If Sample averaging was set to Duplicate, “D” at the end of a sample name indicates the second measurement. If Sample averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis:



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

To copy the data on the Standard Curve(s) tab or Run Chart tab, right-click the plot and choose **Copy to Clipboard**.

Related Topics

Overview

Application Settings

Data Display

Unique Screen Features

Working With Standard Curves

Protein Biuret

Contents

Overview

The Protein Biuret application is similar to the Pierce Modified Lowry application; however, it involves a single incubation and requires more protein for the analysis. The protein-dye complex that is formed in the assay has a deep purple color and is measured at 545 nm. The calibration curve for this assay is linear with standards that range in concentration from 0.5 mg/ml to 10 mg/ml.

Working With Standard Curves

A standard curve is required for colorimetric protein analysis. Here are tips on creating a standard curve:

- A standard curve can be generated using two or more standards.

- The multipoint curve capability allows multiple replicates for each standard. Standards can be run in any order; however, best practice dictates that the standards be measured from the lowest concentration to the highest.
- Determine whether or not to use a standard when analyzing samples against the standard curve.
- Establishing a new standard curve requires creating a new workbook.
- If a previously saved workbook is used, all concentration calculations for newly measured samples will be based on the standard absorbance values saved in the workbook.

Note Each workbook will archive only one standard curve.

Related Topics

Application Settings

Data Display

Unique Screen Features

Making Protein Biuret Assay Measurements

Application Settings

To set data acquisition parameters for a Protein Biuret workbook, click **Settings**.

The follow tabs of settings are available:
Type Tab for Protein Biuret
Measurement Tab for Protein Biuret
Standards Tab for Protein Biuret
Instrument Tab for Protein Biuret
Accessories Tab for Protein Biuret
Samples Tab for Protein Biuret

Related Topics

Overview

Data Display

Unique Screen Features

Making Protein Biuret Assay Measurements

Type Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Type tab in the right pane.

Feature	Description
Name	Application name.
Description (optional)	Description of the template.
Standard curve	Type of quantitative analysis used.
Pathlength	Distance the light travels through the sample.

Related Topics

Measurement Tab for Protein Biuret

Standards Tab for Protein Biuret

Instrument Tab for Protein Biuret

Accessories Tab for Protein Biuret

Samples Tab for Protein Biuret

Measurement Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Measurement tab in the right pane.

Feature	Description
Analysis wavelength	Wavelength(s) to use for the analysis.
Correction	Specifies bichromatic normalization of the absorbance data. • **Single point**. Enter a wavelength value to define the endpoint for the single point baseline. This option generates a baseline correction for each sample measurement by drawing a straight line through the specified baseline point and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance • **Sloping baseline**. Enter two wavelength values to define the endpoints for the sloping baseline. This option generates a baseline correction for each sample measurement by drawing a line between the two specified baseline points and calculating the absorbance value on the drawn baseline at the specified analytical wavelength. The software subtracts the calculated baseline absorbance value from the measured absorbance value and reports the corrected absorbance. • **None**. Uses uncorrected data. Without baseline correction, spectra may be offset from the baseline. If this offset is significant, the calculated protein concentration may be higher than the true value.
Component name	Component to quantify.

Feature	Description
Unit	Component concentration unit. The available units depend on the Formulas & Units tab settings in Options.
Calculate additional results	For selecting or defining formulas for additional data processing, with the results appearing in the analysis report. The formulas are written in a form similar to that used in a command script language, with constants, mathematical functions, etc. All functions are not case-sensitive. Spaces are not allowed between a function name and "(". After adding formulas, edit their information in the formula table as desired. To delete a table row, right-click it and choose **Delete Row**. Some information in the table cannot be modified or deleted. Instructions: ❖ **To add one or more predefined formulas** 1. Click **Select**. 2. For each desired formula, select it in the list and click **Add**. The available formulas depend on the Formulas & Units tab settings in Options. 3. Click **Close**. ❖ **To define a formula** 1. Click **Build**. 2. To enter a formula in its entirety, set **Equation type** to **User defined** and type the equation. To use a provided formula, select it and enter values for the variables. The available formulas depend on the Formulas & Units tab settings. 3. Click **OK**. 4. For selected provided formulas, enter a name and concentration unit in the table. The available units depend on the Formulas & Units tab settings.

Related Topics

Type Tab for Protein Biuret

Standards Tab for Protein Biuret

Instrument Tab for Protein Biuret

Accessories Tab for Protein Biuret

Samples Tab for Protein Biuret

Standards Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Standards tab in the right pane.

Feature	Description
Curve fit type	Type of equation used to create the standard curve from standard concentration values.
Standard averaging	Whether and how to average concentration values from multiple measurements of the same standard or from multiple standards prepared under the same conditions. To average multiple measurements of the same standard, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three similar standards, select **Duplicate** or **Triplicate**, respectively.
Minimum r^2	The r^2 value indicates how well the standard curve fits the standard data points, with 1.0 a perfect fit. If Minimum r^2 is selected, samples can be quantified only after that value is achieved for the standard curve.
Calculate from weight/volume	If available for the current unit, calculates concentration when the weight and volume of material used to prepare each standard is known. Enter the weight and volume for each standard in the appropriate table cells, or enter it later as part of running an analysis.
Use correction factor	If available and selected, specifies a multiplication factor for each standard. Can be used to correct for standard properties (percent purity, water content, etc.) or preparation steps that affect the measured result such as a dilution. When this option is selected, two columns are added to the standards table: Correction Factor and Corrected Concentration. In the Correction Factor column, enter the desired factor for each standard in the table. The values in the Corrected Concentration column are used to create the calibration curve. If a report is generated that includes information about the standards, the report will also include the correction factors and corrected concentrations.
Standards Table	Contains concentration and other information about the standards. To add information from a .csv (comma separated values) file or .tsv (tab separated values) file, click **Import Standards**. To save the information in a .csv file or .tsv file, click **Export Standards**.

Related Topics

Working With Standard Curves

Type Tab for Protein Biuret

Measurement Tab for Protein Biuret

Instrument Tab for Protein Biuret

Accessories Tab for Protein Biuret

Samples Tab for Protein Biuret

Instrument Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Instrument tab in the right pane.

Feature	Description
Mode	Specifies Scan or Fixed data acquisition.
Data mode	Y-axis format for acquired data.
Start wavelength and End wavelength (Scan only)	Starting and ending values of the wavelength range for acquiring data.
Bandwidth	A larger spectral bandwidth increases the amount of energy that passes through the sample. Increasing the bandwidth can improve the signal-to-noise ratio; however, a larger spectral bandwidth decreases the ability to resolve closely spaced spectral features.
Integration time	How long the system acquires and averages data at each data interval (for scanning measurements) or at each measured wavelength (for fixed-wavelength measurements). Increasing the integration time improves the signal-to-noise ratio but reduces the scan speed.
Data interval (Scan only)	Difference in wavelength between two consecutive data points.
Scan speed (Scan only)	Wavelength range covered per unit time. Varies inversely with integration time. Increases as the data interval increases.
Estimated time (Scan only)	Estimated duration of data acquisition. Increases as integration time increases and as the data interval decreases. Varies inversely with scan speed.
Table of wavelengths (Fixed only)	Shows the analysis wavelength(s) entered on the Measurement tab.

Related Topics

Type Tab for Protein Biuret

Measurement Tab for Protein Biuret

Standards Tab for Protein Biuret

Accessories Tab for Protein Biuret

Samples Tab for Protein Biuret

Accessories Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Accessories tab in the right pane. The available parameters depend on the installed accessories. For more information, refer to the user guide for the accessory or search for "Evolution 200 Series Accessories" in the INSIGHT Help system.

The status of accessories can be monitored during measurements. See Instrument Status Monitors.

Related Topics

Type Tab for Protein Biuret

Measurement Tab for Protein Biuret

Standards Tab for Protein Biuret

Instrument Tab for Protein Biuret

Samples Tab for Protein Biuret

Samples Tab for Protein Biuret

Click **Settings** in Protein Biuret to display the Samples tab in the right pane.

Feature	Description
Number of samples	Number of samples in the analysis.
Base name	Beginning of the name for sample spectra, to which a sequential number is appended. For example, the names of three sample spectra using the base name "dye" would be dye1, dye2 and dye3.

Feature	Description
Sample averaging	Whether and how to average concentration values from multiple measurements of the same sample or from multiple samples. To average multiple measurements of the same sample, select **Replicate** and set **Number of replicates** to the total number of measurements. To average values from two or three samples, select **Duplicate** or **Triplicate**, respectively.
Use sample correction factor	If available and selected, specifies a multiplication factor for each sample result. Can be used to correct for sample properties and preparation steps such as a sample dilution that affects the measured result. Enter the desired factor for each sample in the table.
Use weight/volume correction	If available for the current unit, corrects sample concentrations using the entered target weight and volume: corrected concentration = measured concentration * (actual weight / target weight) * (target volume / actual volume)
Use control limits	Displays high and low limit lines on the Run Chart tab to show whether sample concentrations are within the specified limits.
Load Samples	For locating and selecting a .tsv (tab separated values) file or .csv (comma separated values) file containing sample names and descriptions, which are entered in the samples table.
Save Samples	Saves the contents of the samples table in a .tsv (tab separated values) file or .csv (comma separated values) file in a specified location.
Samples table	Lists samples by their names and descriptions, up to a maximum of 1000. To change a sample name, click its cell and edit the text. To enter or change text in a table cell, click it and type. To order the list by sample name or description, click the appropriate column heading. If the table contains just one sample and data is acquired with an accessory that automatically changes samples, sample names are incremented.

Related Topics

Type Tab for Protein Biuret

Measurement Tab for Protein Biuret

Standards Tab for Protein Biuret

Instrument Tab for Protein Biuret

Accessories Tab for Pierce 660 nm Protein Assay

Unique Screen Features

These features are to the left of the spectral display:

Advanced calculations

For selecting predefined formulas for additional data processing, with the results appearing in a calculations table at the bottom of the right pane. Options include basic math and statistics. The formulas can be applied to specific samples and columns of data in the sample measurements table or to selected rows and columns in the calculations table for the current workbook or template. Calculations are applied to all subsequently acquired data. Custom calculations are saved with the workbook or template. Available for all applications except Rate.

To delete the contents of a cell in the calculations table, right-click the cell and choose **Delete**.

❖ To add column and row headings to the calculations table

1. Double-click a cell that needs a heading or other label.
2. In the Cell Properties box, choose **Text**.
3. Type the heading or label text.
4. To select the background color for the text displayed in the calculations table, click **Background**, choose a color and click **OK**.
5. To display the cell contents in bold text, click **Bold Text**.
6. Click **OK**.

❖ To define calculations based on the samples table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.

   The available equations are fixed.
4. Set Source to **Samples table**.
5. Select a sample measurement in the **Measurements** list.
6. Specify the sample rows to include by entering their ID numbers in the **Range/Reference** box.

   Use a colon to specify a range (e.g., 2:5 or 1:N) or commas for individual samples (e.g., 1,3,5).

7. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
8. To display the cell contents in bold text, click **Bold Text**.
9. Choose **OK**.

   The calculated result appears in the selected cell.

❖ To define a calculation based on the calculations table

1. In the calculations table, double-click the cell where you want the calculation result to appear.
2. In the Cell Properties box, choose **Calculation**.
3. Select an **Equation** in the list.
4. Set Source to **Calculations Table**.
5. Specify the table cells to include by entering their IDs in the **Range/Reference** box.

   If Equation is set to **Std. Deviation, Mean**, or **%RSD**, use a colon to specify a range (e.g., A2:A5 or A1:AN) or commas for individual cells (e.g., A1,A3,A5).

   If Equation is set to **Addition**, **Subtraction**, **Multiplication**, or **Division**, specify one cell location in each Reference box. (e.g., A1).

   If Equation is set to a **Factor**, use the first (Reference) box to specify a cell location and the second (Factor) box to enter the factor.
6. To select the background color for the cell that displays the calculated result, click **Background**, choose a color and click **OK**.
7. To display the cell contents in bold text, click **Bold Text**.
8. Choose **OK**.

   The calculated result appears in the selected cell.

Related Topics

Overview

Application Settings

Data Display

Unique Screen Features

Making Protein Biuret Assay Measurements

Making Protein Biuret Assay Measurements

See Sample and Standards Preparation for more information.

❖ To make Protein Biuret assay measurements

1. Click **Measure Protein Biuret** in Protein Biuret.

Note To start an analysis immediately after viewing or changing the template settings, click the **Measure** action button instead and skip to step 3.

2. If standards need to be acquired, click **Measure**. If standards have already been acquired, skip to step 7 to acquire sample data.

3. Follow the instructions that appear.

   Use the appropriate buffer for the blank. See the reagent directions for details.

4. When the standards information appears, modify it if desired.

   More:

   If Standard averaging on the Standards tab in Settings was set to Duplicate, “D” at the end of a standard name indicates the second measurement to be made of the standard. If Sample averaging was set to Triplicate, “D” and “T” at the end of standard names indicate the second and third measurements to be made, respectively.

   To enter previously saved standards information, use **Import Standards**.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each standard in the table.

   To remove a standard, select it, right-click the table and choose **Clear Standard**. To remove all the standards, choose **Clear Table**.

   To save standards information for later use, use **Export Standards**.

5. Click **Continue**.

6. Follow the instructions that appear, installing the specified standards.

   The Standard Curve(s) tab displays the resulting standard curve (or curves). Specify the standards to use for the curve (or curves) by selecting **Yes** or **No** in the **Use** column in the table.

   More:

Method Uncalibrated

If a standard curve is not calibrated...

- Try selecting a different curve fit type.
- Try remeasuring a standard using the correct standard material: select the listed standard, right-click the table and choose **Remeasure**.
- Try changing the setting of **Minimum r^2** on the Standards tab and acquire the standards again in a new workbook.

Method Calibrated

This is only an indicator that the required minimum number of points have been established for the selected curve fit type. It does not validate the integrity of the curve. For example, additional standards may be required to cover the expected assay concentration range.

The table contains the columns of information specified on the Configuration tab in Reports. Examples include sample identification, user name, and the results of replicate, duplicate or triplicate measurements and their standard deviation.

If Standard averaging was set to Duplicate, “D” at the end of a standard name indicates the second measurement. If Standard averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

To permanently remove an acquired standard from the analysis, right-click it and choose **Remove**. Its information is crossed out but not removed from the table.

The Data tab displays the acquired data (a fixed data point or scan spectrum) for the standard selected in the table. To access commands for customizing the display, including adding annotation, right-click the data. See Data Display for more information.

7. Click **Measure**.

8. Follow the instructions that appear.
9. When samples information appears, modify it if desired.

   If Calculate from weight/volume was selected on the Standards tab, enter the weight and volume for each sample in the table.

   More:

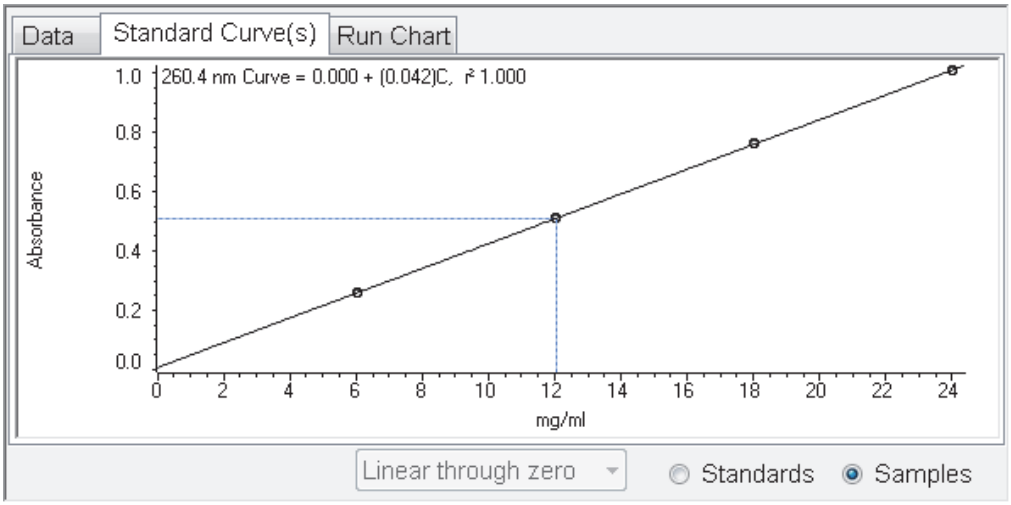
   If Sample averaging on the Samples tab in Settings was set to Duplicate, “D” at the end of a sample name indicates the second measurement to be made of the sample. If Sample averaging was set to Triplicate, “D” and “T” at the end of sample names indicate the second and third measurements to be made, respectively.

To enter previously saved samples information, use **Load Samples**. To save the samples information, use **Save Samples**.

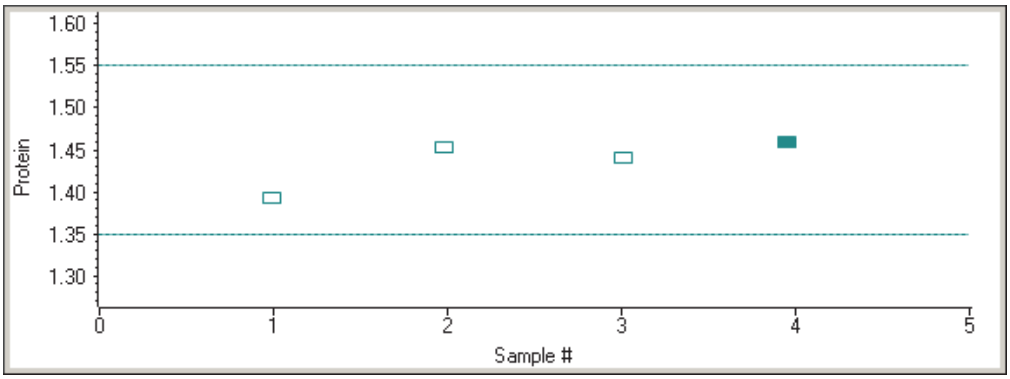
10. If only one sample will be measured, install it.
11. Click **Continue**.
12. Follow any instructions that appear, such as to install a specified sample.

The sample results table contains the columns of information specified on the Configuration tab in Reports. If Sample averaging was set to Duplicate, “D” at the end of a sample name indicates the second measurement. If Sample averaging was set to Triplicate, “D” and “T” indicate the second and third measurements, respectively.

The Standard Curve(s) tab shows graphically the relationship between the standard curve, measured spectral intensity and calculated concentration for the selected sample: A horizontal line connects the sample spectral intensity value on the Y-axis to the standard curve. A vertical line connects that point to the sample concentration value on the X-axis:



The Run Chart tab plots the concentration of the measured component versus sample number. If Use concentration limits was selected on the Samples tab, horizontal limit lines show whether the concentrations are within the specified limits:



To measure a sample again, right-click its row in the results table and choose **Remeasure**. After the remeasurement, the previous information for the sample is crossed out (but not removed from the table).

To copy the data on the Standard Curve(s) tab or Run Chart tab, right-click the plot and choose **Copy to Clipboard**.

Related Topics

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Performance Verification

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Overview of PV

Use **Performance Verification** to set up and run tests to check the performance of the instrument. Individual performance tests can be run manually or automatically using a compatible CVC (Calibrated Validation Carousel) accessory. For information about the available CVC configurations, refer to the *CVC User Guide* in the "Accessories" section or your documentation media.

Click here for information on running the performance tests. Test results are displayed in reports that are saved automatically and can be opened later (see My Data).

PV Test Descriptions

This section describes the performance tests that can be run manually if you have the required standards. Many of the tests described here may also be included in the CVC test suites. For information about the test samples included with each CVC and the corresponding test suite, refer to the to the *CVC User Guide* in the "Accessories" section or your documentation media.

Test	Description
Wavelength Accuracy (Hg lamp)	Locates the peaks from a mercury lamp accessory and displays their measured and allowed range of wavelengths. A mercury lamp has strong, fundamental lines throughout the UV-visible range. These emission lines are a property of the lamp and serve as a fundamental wavelength standard that does not require calibration. The wavelengths and tolerance values cannot be changed. To run this test, use an empty cell holder and install the Mercury Lamp calibration accessory properly in the instrument. For more information, refer to the *Mercury Lamp User Guide* in the "Accessories" section of your documentation set.
Wavelength Repeatability (Hg lamp)	Measures the ability of the spectrophotometer to return to an identical wavelength in a repeatable manner. The test uses a mercury lamp accessory, which has a strong, fundamental line near 546 nm. This emission line is an intrinsic property of the lamp and serves as a fundamental wavelength standard that does not require calibration. The wavelength and tolerance values cannot be changed. To run this test, use an empty cell holder and install the Mercury Lamp calibration accessory properly in the instrument. For more information, refer to the *Mercury Lamp User Guide* in the "Accessories" section of your documentation set.
Wavelength Accuracy (Xe lamp)	Locates the peak near 542 nm of the internal xenon lamp and displays the measured and allowed range of wavelengths. A xenon lamp has strong, fundamental lines throughout the UV-visible range. These are an intrinsic property of the lamp and serve as a fundamental wavelength standard that does not require calibration. The wavelength and tolerance values cannot be changed. To run this test, use an empty cell holder.

Test	Description
Wavelength Repeatability (Xe lamp)	Measures the ability of the spectrophotometer to return to an identical wavelength in a repeatable manner. The test uses the internal xenon lamp, which has a strong, fundamental emission line near 542 nm. This line is a property of the lamp and serves as a fundamental wavelength standard that does not require calibration. The wavelength and tolerance values cannot be changed. To run this test, use an empty cell holder.
Wavelength Accuracy (Holmium glass, CP)	Locates the peaks of a holmium glass standard and displays their measured and allowed range of wavelengths as defined in the *Chinese Pharmacopeia* for this standard. The wavelengths and tolerance values are predefined and cannot be changed. To run this test, install a holmium glass standard in the instrument.
Wavelength Accuracy (Holmium oxide liquid)	Locates the peaks of a holmium oxide standard and displays their measured and allowed range of wavelengths. A holmium oxide solution prepared in dilute perchloric acid has well defined absorption peaks throughout the UV-visible range. These peaks are a property of the material and serve as a wavelength standard. The wavelengths and tolerance values cannot be changed. To run this test, install a holmium oxide liquid standard prepared in dilute perchloric acid.
Wavelength Repeatability (customized)	Measures the ability of the spectrophotometer to return to an identical wavelength in a repeatable manner using an appropriate standard. For example, a holmium oxide solution prepared in dilute perchloric acid has well defined absorption peaks throughout the UV-visible range. To run this test, install a wavelength repeatability standard and enter its wavelength, absorbance and tolerance values in the boxes to the right of the test name. For more information, refer to the documentation that came with the standard.

Test	Description
Photometric Accuracy (customized)	Automates the comparison of measured absorbance (or %T) of photometric accuracy standards with their certified values. To run this test, install a photometric accuracy standard that has been calibrated to known absorbance values at specified wavelengths and enter its wavelength, absorbance and tolerance values in the boxes to the right of the test name.
Photometric Repeatability (customized)	Automates 10 absorbance (or % T) measurements of a photometric repeatability standard at a predefined wavelength and calculates the standard deviation of the measurements. Generates a Pass result when the standard deviation is less than 0.001. To run this test, install a photometric repeatability standard that has been calibrated to known absorbance values at specified wavelengths and enter its wavelength, absorbance and tolerance values in the boxes to the right of the test name.
Resolution (Toluene/Hexane)	Measures the ability of the spectrophotometer to resolve adjacent features in a spectrum. To run this test, install a 0.02% (v/v) solution of toluene in hexane and a hexane blank. The wavelengths and tolerance values cannot be changed.
Stray Light , • KCl, 198 nm • KI, 220 nm • NaI, 220 nm • NaNO2, 340 nm	Compares measured stray light at the indicated wavelength with the allowed range of values. To run this test, install a stray light standard designed to measure stray light at one of the available wavelengths. The wavelength and tolerance values cannot be changed.
Photometric Noise • 0A (260 nm) • 1A (260 nm • 2A (260 nm)	Measures the amount of noise at 260 nm. To run the 0A (260 nm) noise test, use an empty cell holder. For the latter two tests, use 1A and 2A metal-on-quartz filters suitable for the UV region for these tests.

Test	Description
Baseline Flatness (Abs, 800-200 nm)	Measures the flatness of a baseline scan over the region from 800 nm to 200 nm with smoothing applied to the data. To run this test, use an empty cell holder.
Photometric Drift	Measures the absorbance drift of the instrument over a one-hour period. To run this test, use an empty cell holder. Let the instrument warm up fully before initiating this test.

Related Topics

Overview of PV

Performing PV Tests

Performing PV Tests

❖ **To perform PV tests**

1. If using a CVC, install the CVC in the spectrophotometer sample compartment.

   For information, refer to the to the *CVC User Guide* in the "Accessories" section or your documentation media.

1. Click **Performance Verification** in the right pane in Home.

   Note Select the **Classic** group in Home if Performance Verification is not available for the current group.

   The available tests appear in the right pane. If no CVC is installed, all of the tests described in the previous section will be available. If a compatible CVC is properly installed and a valid calibration file has been loaded, the Performance Verification screen will display the names of the performance tests that CVC is designed to run. Refer to Calibrated Validation Carousels (CVCs) for more information.

   Note Tests that require the Mercury Lamp calibration accessory can be selected only after the accessory has been properly connected and installed in the instrument.

2. Select the tests to run.

   To select or deselect all the listed tests, double-click **Run** above the column of check boxes.

3. For selected tests that require it, enter information such as wavelength, value and tolerance in the provided boxes.

Heed the following precaution if using an accessory:

CAUTION Avoid pinch hazard. If using a CVC accessory, keep hands and objects clear of the accessory during operation.

4. Click the **Run** button in the left pane.

5. Follow the instructions that appear.

   To end the tests before they are finished, click **Stop**. Alternatively, click **Cancel** when a prompt appears during a test.

   The test results appear in a window. The test results can be printed or copied from the window. A signature block appears at the end.

   Completed PV reports are saved automatically and appear on the PV Reports tab in My Data. See My Data for instructions for viewing results later.

6. Click **Close**.

Related Topics

Overview of PV

PV Test Descriptions

INSIGHT Security Software

Optional INSIGHT Security software adds features for digitally signing files and verifying digital signatures, as explained in this document or Help system.

INSIGHT Security software, together with Thermo Security Administration software, also lets the system administrator set system policies and control user access to INSIGHT software features. See Access Control, System Policies, and Signature Meanings for complete information.

NOTICE You must install Security Administration software and associate INSIGHT Security software with it before running INSIGHT Security software for the first time.

Contents

Getting Started with INSIGHT Security Software

The steps for setting up a secure system are outlined below. For more detailed instructions, read the indicated sections of the manuals on the documentation media for your spectrophotometer and INSIGHT software.

1. Set up Windows® administration including accounts, groups and other aspects of Windows administration—performed by the network administrator or system administrator. Include at least one user with full administrative rights and one with restricted rights. For more information, refer to the "Setting Up Windows Administration" chapter of the *Security Administration User Guide*.

2. Install Security Administration software and add INSIGHT Security software to the security database—performed by the system administrator or lab manager. Security Administration can be installed on the computer running INSIGHT Security software or on a computer that will be the server for Security Administration (recommended if multiple computers will run INSIGHT Security software).

   To add the INSIGHT Security application to the security database:

   a. Open the Security Administration program.

   b. Choose **File** (menu) > **Add Application**.

   c. Select the INSIGHT.XML file. The file is found on the Root directory of the software installation media.

   d. If you will be installing other add-on software such as CUE that require access control, repeat steps a through c to add their XML files (for example CUE.XML) to the security database.

   e. Restart the computer that has INSIGHT Security software installed, search for the server and then test the connection.

   f. Start INSIGHT Security software and complete the hardware installation of the spectrophotometer. (If you are running workstation software, this step is not needed.)

3. Install INSIGHT Security Software on each computer that will be used to run INSIGHT software—performed by the system administrator or lab manager. Any other add-on software you purchased can also be installed at this time.

   If you are using a network, after installing INSIGHT software, set the Thermo Fisher Scientific service accounts so that they are not running as local system accounts. Refer to the "Setting Service Accounts" chapter of the *Security Administration User Guide* for more information.

4. Set up INSIGHT software accounts and file permissions—performed by a system administrator or lab manager familiar with INSIGHT software. For more information, refer to the "Setting Up Client Application Accounts" chapter of the *Security Administration User Guide* and Access Control, System Policies, and Signature Meanings in this document.

Access Control, System Policies, and Signature Meanings

The Access Control features, System Policies and Signature Meanings described below are available in Security Administration for INSIGHT software when INSIGHT Security software is installed and added to the security database.

Use the Access Control and System Policy features to set the rights of individual users or groups of users to use the protected features of INSIGHT software (the features included in the security database). A protected feature will be available only if the logged-in user has the right to use it.

Use the Signature Meanings features to specify the meanings that will be available for electronic signatures for each user or user group. For example, the Signature Meanings can be set so that only a particular user—for instance, the lab manager—is allowed to sign a file with the "Approval" meaning.

❖ To set access control features, system policies and signature meanings for INSIGHT

1. Start the Security Administration program.
2. **Open the Access Control folder** for INSIGHT by clicking its plus sign.

   A tree of folders and other items appears. Each item in the tree represents a protected function or group of functions for which access control is available.
3. Click a feature to display options for controlling access to that feature. For more information about a feature, see Table 1.
4. **Open the System Policies folder** for INSIGHT by clicking its plus sign.

   A tree of icons appears. Each icon in the tree represents a system policy or group of policies; that is, operations for which access control is available.
5. If a check box appears to the left of a policy, you can specify whether it is selected or not selected for different "policy groups." A policy group is a group of users for whom you can set system policies. For more information, refer to the "Creating a policy group" chapter of the *Security Administration User Guide*." Other policies let you specify system attributes such as a default configuration or default directory.
6. **Open the Signature Meanings folder** for INSIGHT by clicking its plus sign.

   The first time Security Administration is run, the default list of signature meanings appears. See Table 1 for descriptions of the default signature meanings for INSIGHT software.
7. Click a signature meaning in the list to display information about its access rights. If a check mark appears to the left of a listed user group, those users can select that signature meaning when signing a file.

   If changes have been made to the list, the available meanings in INSIGHT software may be different. Refer to the "Changing the Available Signature Meanings" chapter of the *Security Administration User Guide* for more information.
8. When you are finished setting access options for all features and policies, choose **File** > **Save** to save the settings in the security database.

Note If a client application was running while you used Security Administration to change its security policy settings, the new settings will not take effect until the application is exited and restarted.

For more information, refer to the "Setting Security Policies for Client Applications" chapter of the *Security Administration User Guide*.

The table below describes INSIGHT access control features and policies.

Table 1. INSIGHT access control features and policies

Feature	Description
Ability to run INSIGHT	Specifies which users can run INSIGHT software. (See "Controlling access to client application features" in the "Using Security Administration" chapter of the *Security Administration User Guide* for general instructions.) If a user has not been granted the ability to run the software, an error message appears when the user attempts to start it.
Ability to delete workbooks	Specifies which users can delete workbook files with the Delete button in My Data.
Ability to delete PV reports	Specifies which users can delete Performance Verification reports with the Delete button in My Data.
Ability to delete templates	Specifies which users can delete template files with the Delete button in My Data.
Ability to delete Scan samples	Specifies which users can delete Scan sample data files from the current workbook by right-clicking a sample in the results table in Measure Scan and choosing Remove.
Ability to edit templates	Specifies which users can set parameters in Settings.
Ability to configure reports	Specifies which users can change settings in Reports.
Ability to perform PV tests	Specifies which users can run Performance Verification tests.
Ability to perform wavelength calibration	Specifies which users can use the Wavelength Calibration buttons on the Calibrations tab in System Settings.
Ability to calibrate Sipper	Specifies which users can use the Sipper Calibrate button on the Calibrations tab in System Settings.
Ability to calibrate Linear Cell Changer	Specifies which users can use the Linear Cell Changer Calibrate button on the Calibrations tab in System Settings.
Ability to update CVC calibration	Specifies which users can use the Load CVC Calibration button in Performance Verification.
Ability to select CVC tests to perform in PV	Specifies which users can select listed tests for the CVC in Performance Verification.
Math menu	Specifies which users can use the commands in the Math menu.
Analyze menu	Specifies which users can use the commands in the Analyze menu.
Ability to set Rate parameters	Specifies which users can set parameters in Settings for the Rate application.

Feature	Description
Options tabs	Specifies which users can change settings on the tabs in Options.
System Settings tabs	Specifies which users can change settings on the tabs in System Settings.
Authenticate on startup	Requires users in the specified policy groups to enter a valid name and password when starting INSIGHT software.
Prevent overwriting of files	Ensures that existing files are not overwritten when users in the specified policy groups save workbook or template files. If a user for whom this policy is selected attempts to save a file using the same file name as an existing file, a message says the file cannot be overwritten. The user can choose OK to close the message and then save the file using a different file name. If a user saves a file while this policy is selected, the file will be set to read-only in its properties. In the future, users will not be able to overwrite this file, even when this policy is not selected.
Prevent delete, rename and right-click in file dialog boxes	Prevents users in the specified policy groups from performing these operations in file dialog boxes: • Deleting a file by selecting it and pressing the Delete key on the keyboard. • Renaming a file by selecting its name (by slowly clicking the name twice) and typing a new name. • Displaying a shortcut menu by right-clicking a file. Normally a menu displayed this way lets the user perform operations such as copying, deleting or renaming the file. This policy can be used in conjunction with the Prevent changing directories when saving files policy (see below) to achieve the desired type of control over file operations for each user.
Prevent printing unsaved data	Prevents users in the specified policy groups from printing spectral data that has not been saved.
Prevent changing directories when saving files	Prevents users in the specified policy groups from changing directories (“browsing”) when saving files.

Feature	Description
Prevent signature reason entry when signing files	Prevents users in the specified policy groups from entering a custom signature meaning when signing a file. When this policy is selected, only the standard signature meanings are available for the affected users. When this policy is not selected, the prompt requesting a signature lets the user type a meaning in the Reason For Signature text box.
Require signature when saving workbook files	Requires users in the specified policy groups to provide a digital signature when saving workbooks with Save Workbook or Save As Workbook in the File menu. If this policy is not selected, users in the specified policy group must use Security (menu) > Sign > Active Workbook or Workbook File to sign a workbook after it has been saved.
Require signature when saving template files	Requires users in the specified policy groups to provide a digital signature when saving templates with Save Workbook Settings as Template in the File menu. If this policy is not selected, users in the specified policy group must use Security (menu) > Sign > Template File to sign a workbook after it has been saved.
Require signature when saving PV results	Requires users in the specified policy groups to provide a digital signature when saving Performance Verification results.
Directory for workbooks	Designates for each policy group a directory for saving workbooks with Save Workbook or Save As Workbook in the File menu.
Directory for templates	Designates for each policy group a directory for saving templates with Save Workbook Settings as Template in the File menu.

Feature	Description
Directory for PV reports	Designates for each policy group a directory for automatically saving Performance Verification reports.
Allow access only to listed button groups (blank field allows access to all groups)	Specifies the INSIGHT software user groups that are available to users in the specified policy group. Selecting a user group in Home displays the application buttons for that group. See Applications Tab for more information. Type the desired user groups in the text box in the Description box, with a comma and a space between them; for example: `Classic, Quality Control` In this example, these users would have access to the applications for the Classic group (Fixed, Scan, Quant, Rate, Bio Applications and Performance Verification) and the applications for the Quality Control group. Leave the text box blank to give users in the specified policy group access to all applications.

The signature meanings in the default list for INSIGHT software are described below.

Signature Meaning	Description
Authorship - signifies ownership	Indicates that the user signing the file is the person who created it.
Approval - the record is approved for use	Indicates that the user signing the file has approved it for use.
Reviewed - record contents have been reviewed	Indicates that the user signing the file has reviewed it.
Revision - the record has been revised	Indicates that the user signing the file has changed it.

Note Users can be allowed to enter custom signature meanings. See "Prevent signature reason entry when signing files" in Access Control, System Policies, and Signature Meanings for details.

Event Logging

To provide a system audit trail, Security Administration uses the Event Log service in Windows software to record Security Administration and client application operations, or "events," in a log that you can view with Event Viewer. Examples of logged events include the date the event occurred, the type of event, and the user name of the person who was logged in

when the event occurred. The Event Log service allows all file operations to be logged, both within and outside of applications that are run on the system, even if a client application is not running. By recording this information, Security Administration allows your system to meet the audit trail requirements of 21 CFR Part 11.

❖ **To view the log of events for Security Administration and your client applications**

1. Start the Windows Event Viewer on the computer where Security Administration is installed.

   Refer to your Windows documentation for information about locating the Event Viewer feature in your version of Windows software.

2. In the left pane of the Event View window, click the Thermo Electron icon.

   A log of significant events that occurred while your client applications were being used appears in the right pane. You can sort the events according to date, category, user and so on by clicking the column headings.

3. To see detailed information about an event, double-click the event (use the up and down arrows to see information about the previous or next event).

   You can also export the list of events.

4. When finished, close the Event Viewer.

For more information on the Windows Event Viewer, see its associated Help system.

Sample History Information

To access information about a data point or spectrum, open the workbook, right-click its row in the sample measurements table and choose **Properties**. Click the tabs along the top of the Sample Properties window to see information about the sample, the instrument and any accessories that were used to collect the data, and any electronic signatures that were applied.

INSIGHT Security Software Icons


This icon at the bottom of the INSIGHT window indicates that the current workbook is signed.


This icon indicates that current workbook has not been signed since it was last changed.


This icon indicates that current workbook has not been saved (or signed).

To see information about the signature status of the current workbook, click any of these icons.

When INSIGHT Security software is installed...

- The root directory for template groups shown on the Preference tab in Options is set using Thermo Security Administration.
- A Sign button appears in the Performance Verification report window. Use it to sign the displayed report.

Related Topics

Signing a File

Verifying a Signature

Signing a File

If INSIGHT Security Software is installed, use the following procedures to digitally sign workbook and template files. The visible portion of a digital signature consists of a user name, a date and a stated reason for signing. A digital signature also contains encrypted information used to detect whether the file has changed since it was signed.

❖ To sign the open workbook

1. Choose **Security** (menu) > **Sign** > **Active Workbook**.
2. Enter a password.
3. Select a signature reason (if permitted).
4. Choose **OK**.

   A verification message appears.
5. Choose **OK**.

❖ To sign a saved workbook

1. Choose **Security** (menu) > **Sign** > **Workbook File**.
2. Locate and select the desired file and then click **Open**.
3. Enter a password.
4. Select a signature reason (if permitted).
5. Choose **OK**.

   A verification message appears.
6. Choose **OK**.

❖ **To sign a saved template**

1. Choose **Security** (menu) > **Sign** > **Template File**.
2. Locate and select the desired file and then click **Open**.
3. Enter a password.
4. Select a signature reason (if permitted).
5. Choose **OK**.

   A verification message appears.
6. Choose **OK**.

Related Topics

INSIGHT Security Software

Verifying a Signature

Verifying a Signature

If INSIGHT Security Software is installed, use the following procedures to verify digital signatures for workbook and template files.

❖ **To verify the signature for a workbook**

1. Choose **Security** (menu) > **Verify Signature** > **Workbook File**.
2. Locate and select the desired file and then click **Open**.

   A verification message appears.
3. Choose **OK**.

❖ **To verify the signature for a template**

1. Choose **Security** (menu) > **Verify Signature** > **Template File**.
2. Locate and select the desired file and then click **Open**.

   A verification message appears.
3. Choose **OK**.

Related Topics

INSIGHT Security Software

Signing a File

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