Mobile Phase Chemical Name	Formula	Concentration	Volume or Mass (per 1 L)	Preparation Procedure Number*	pH Adjustment Acid/Base	MS Compatible?
Acetic Acid	CH_3COOH	0.1%	1.0 mL	1	--	Yes
Ammonium Hydroxide	NH_4OH	0.1%	1.0 mL	1	--	Yes
Ammonium Hydroxide	NH_4OH	0.2%	2.0 mL	1	--	Yes
Ammonium Hydroxide	NH_4OH	1.0%	10.0 mL	1	--	Yes
Ammonium Hydroxide	NH_4OH	100 mM	6.9 mL	1	--	Yes
Formic Acid	HCOOH	0.05%	0.5 mL	1	--	Yes
Formic Acid	HCOOH	0.1%	1.0 mL	1	--	Yes
Formic Acid	HCOOH	0.2%	2.0 mL	1	--	Yes
Formic Acid	HCOOH	0.5%	5.0 mL	1	--	Yes
Formic Acid	HCOOH	50 mM	2.1 mL	1	--	Yes
Formic Acid	HCOOH	100 mM	4.2 mL	1	--	Yes
Phosphoric Acid	H_3PO_4	0.1%	1.0 mL	1	--	No
Trifluoroacetic acid (TFA)	CF_3COOH	10 mM	0.8 mL	1	--	Yes[3]
Trifluoroacetic acid (TFA)	CF_3COOH	0.1%	1.0 mL	1	--	Yes[3]
Acetic Acid[2] Triethylamine (TEA)[2] EDTA	CH_3COOH $(CH_3CH_2)_3N$ $C_{10}H_{14}N_2Na_2O_8{\cdot}2H_2O$	50 mM CH_3COOH 50 mM TEA 2 mM EDTA	2.8 mL 6.9 mL 0.75 g	2	CH_3COOH or $(CH_3CH_2)_3N$	No
Acetic Acid[2] Triethylamine (TEA)[2]	CH_3COOH $(CH_3CH_2)_3N$	100 mM CH_3COOH 100 mM TEA	5.6 mL 13.9 mL	3	CH_3COOH or $(CH_3CH_2)_3N$	Yes
Ammonium Bicarbonate	NH_4HCO_3	5 mM	0.40 g	2	HCOOH or NH_4OH	Yes
Ammonium Bicarbonate	NH_4HCO_3	10 mM	0.79 g	2	HCOOH or NH_4OH	Yes
Ammonium Bicarbonate	NH_4HCO_3	20 mM	1.58 g	2	HCOOH or NH_4OH	Yes
Ammonium Bicarbonate	NH_4HCO_3	100 mM	7.91 g	2	HCOOH or NH_4OH	Yes
Ammonium Formate	NH_4COOH	10 mM	0.63 g	2	HCOOH	Yes
Ammonium Formate	NH_4COOH	15 mM	0.95 g	2	HCOOH	Yes
Ammonium Formate	NH_4COOH	100 mM	6.31 g	2	HCOOH	Yes
Ammonium Acetate	CH_3COONH_4	10 mM	0.77 g	2	CH_3COOH	Yes
Ammonium Acetate	CH_3COONH_4	20 mM	1.54 g	2	CH_3COOH	Yes
Ammonium Acetate	CH_3COONH_4	100 mM	7.71 g	2	CH_3COOH	Yes
Hexafluoroisopropanol (HFIP) Triethylamine (TEA)	C_2F_6CHOH $(CH_3CH2)_3N$	0.4 M HFIP 16.3 mM TEA	41.5 mL 2.3 mL	4	TEA	Yes
Sodium Phosphate, Dibasic	Na_2HPO_4	20 mM	2.84 g	2	H_3PO_4	No

(1) – See specified preparation procedure on next page
(2) – Triethylammonium Acetate (Acetic Acid + Triethylamine → Triethylammonium Acetate)
(3) – Can suppress MS signal at higher concentrations

Preparation Procedure 1

(1) Add the indicated amount(s) of mobile phase additive(s) to 950 mL of water.
(2) Mix solution thoroughly.
(3) Measure, adjust and record mobile phase pH (if desired).
(4) Add water to final volume of 1 L, degas and transfer to mobile phase container.

Preparation Procedure 2

(1) Add indicated amounts of buffers to 400 mL of water and mix thoroughly until all salts are dissolved.
(2) Filter solution through a 0.2µm HPLC-certified Nylon filter (e.g., WAT200533).
(3) Add water to 950 mL and check pH.
(4) Adjust pH to desired value.
(5) Add water to final volume of 1 L, degas and transfer to mobile phase container.

Preparation Procedure 3*

(1) Add the indicated amounts of mobile phase buffers to 950 mL of water.
(2) Mix TEAA buffer solution thoroughly, measure pH, and adjust pH up ($(CH_3CH_2)_3N$) or down (CH_3COOH) to desired value.
(3) Add water to final volume of 1 L. Use this 100 mM TEAA buffer for mobile phase preparation described in step (4).
(4) Combine 100 mM TEAA buffer prepared in previous step (3) with organic modifier (e.g., for a 95% 100 mM TEAA / 5% ACN mobile phase (v:v), mix 950 mL of 100 mM TEAA buffer with 50 mL ACN).
(5) Degas and transfer to mobile phase container.

Preparation Procedure 4*

(1) Add the indicated amounts of mobile phase buffers to 950 mL of water.
(2) Mix buffer solution thoroughly, measure pH, and adjust if necessary with TEA.
(3) Add water to final volume of 1 L. Use this buffer for mobile phase preparation described in step (4).
(4) Combine buffer prepared in previous step (3) with organic modifier (e.g., for a 95% 0.4 M HFIP, 16.3 mM TEA / 5% MeOH mobile phase (v:v), mix 950 mL of buffer with 50 mL MeOH).
(5) Degas and transfer to mobile phase container.

(*) – Preparation Procedures 3 and 4 involve premixing the aqueous and organic portions of the mobile phases. These mobile phases are mixed volume:volume (v:v) and unlike the other preparation procedures described here, are not diluted to final volume. To learn more about HPLC separation procedures for oligonucleotide analysis and purification, please refer to the *XTerra® Columns Oligonucleotide Applications Notebook* (Literature Code 720000396EN).