RNA Sample Preparation & High-Throughput Purification for TBDMS & TOM Chemistries Using Clarity® QSP™

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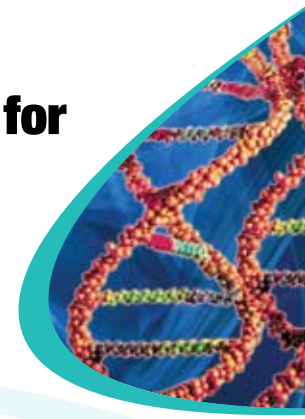


Introduction

The recent biological and therapeutic discoveries of RNA have led to numerous alterations in the chemical synthesis of oligoribonucleotides. Adapted from DNA chemistry, RNA synthesis requires the delicate balance of securing 2' hydroxyl protection while providing incremental release of the 5' protecting group. The prevalent commercial synthetic mechanism features fluoride-labile 2' protection in conjunction with an acid-labile 5' ether.

For years, t-butyldimethylsilyl (TBDMS) chemistry has been the mainstay for 2' deprotection during RNA assembly. While commonly used, noteworthy downsides of TBDMS chemistry include low coupling yields, restricted sequence length, and alkaline induced 2' to 3' isomerization. Offering to improve synthetic RNA efficiencies and ultimately yields, modern advances have produced alternative chemistries for 2' protection most notably triisopropylsilyloxymethyl (TOM). The newly developed platform provides superior alkaline stability to eliminate 2' to 3' isomerization, and offers greater coupling yields by evading steric hindrance.

To better accommodate improved changes in synthetic designs, post-synthesis revisions are necessary to ensure efficient deprotection and purification. While Clarity QSP can effectively purify any crude synthetic RNA sample regardless of the chemistry, QSP is not a universal technique and tailored sample preparation may be warranted for optimal results. The following serves as guide for 2' cleavage and RNA sample preparation prior to trityl-on QSP cartridge or high-throughput purification.

Sample Preparation & Clarity QSP Purification for RNA-TBDMS Chemistry (t-butyldimethylsilyl)

IMPORTANT: *Please note that for all trityl-on RNA purification it is imperative that the final 5' DMT group be retained following synthesis completion.*

TBDMS Deprotection

(Wincott, et al. Nucleic Acids Research, 1995)

Support cleavage and primary deprotection:

- Add an appropriate volume of EtOH: NH_4OH (1:3) to CPG column according to synthesis scale. Typically, the volume used is 150 µL per 100 nmole
- Allow 17 hours for room temperature incubation and 2 hours at 55 °C
- Evaporate to dryness
- Prepare fresh RNA deprotecting cocktail in volumes according to the below table
- Always add TEA.3HF last to prevent coagulation of the mixture

2' Deprotection & Synthesis Scale

Reagents	≤200 nmole	1 µmole	10 µmole	20 µmole	50 µmole
N-Methylpyrrolidone	1500 µL	1500 µL	7.5 mL	7.5 mL	7.5 mL
Triethylamine	750 µL	750 µL	3.75 mL	3.75 mL	3.75 mL
2-Triethylamine-trihydrofluoride (TEA.3HF)	1000 µL	1000 µL	5 mL	5 mL	5 mL
Number of Oligos	**32**	**12**	**5**	**2**	**1**

Support cleavage and primary deprotection (cont'd)

- Using the below scale-volume chart add 2' deprotecting solution to RNA pellet
- Vortex / mix briefly
- Heat @ 65 °C for 1.5 hrs / (agitation optional)
- Cool to room temperature
- In accordance with scale-volume chart slowly add volume of 1.5 M ammonium bicarbonate to quench
- Add an equal volume of Clarity QSP RNA-TBDMS loading buffer to quenched deprotection solution

Clarity QSP Sample Preparation & Synthesis Scale

Synthesis Scale	2' Deprotection Cocktail	Na_2HCO_3	Clarity QSP RNA-TBDMS Loading Buffer	Total Volume
≤200 nmole	100 µL	400 µL	500 µL	1 mL
1 µmole	250 µL	1 mL	1.25 mL	2.5 mL
10 µmole	2.50 mL	7.5 mL	10 mL	20 mL
20 µmole	5.0 mL	15 mL	20 mL	40 mL
50 µmole	12.5 mL	37.5	50 mL	100 mL

QSP Purification for ≤0.2 µmole

Formats: 50 mg/ 96-Well Plate (Part No.: 8E-S102-DGB)
50 mg/ 1 mL Cartridge (Part No.: 8B-S102-DAK)

Purification Protocol:

- Add equal volume of Clarity QSP RNA-TBDMS loading buffer (500 µL) to RNA sample, total volume: 1 mL
- Condition: 1 mL MeOH (0.5 mL x 2)
- Equilibrate: 1 mL Water (0.5 mL x 2)
- Load Vol of RNA Oligo
- Detritylate: 1 mL 2 % DCA or TFA
- *Rinse: 1 mL Water (0.5 mL x 2)
- Dry sorbent @ 10" Hg (~1 min)
- *Elute: 1 mL 20 mM Na_2CO_3 / 50 % Methanol

QSP Purification for 1.0 µmole

Format: 150 mg/ 3 mL Cartridge (Part No.: 8B-S102-SBJ)

Purification Protocol:

- Add equal volume of Clarity QSP RNA-TBDMS loading buffer (1.25 µL) to RNA sample, total volume: 2.5 mL
- Condition: 3 mL MeOH (1.5 mL x 2)
- Equilibrate: 3 mL Water (1.5 mL x 2)
- Load Vol of RNA Oligo
- Detritylate: 1.5 mL 2 % DCA or TFA
- *Rinse: 2 mL Water (1 mL x 2)
- Dry sorbent @ 10" Hg (~1 min)
- *Elute: 2 mL 20 mM Na_2CO_3 / 50 % Methanol

*For users that avoid any salts in the final elution, a 1 mL rinse using a 20 mM concentration of an aqueous buffer (Na, NH_4 or tris) can be used in the rinse step. The RNA can then be eluted in a water/ methanol solution. To avoid potential depurination, it is recommended that the pH of the final solution be that of a physiological pH (7-8).

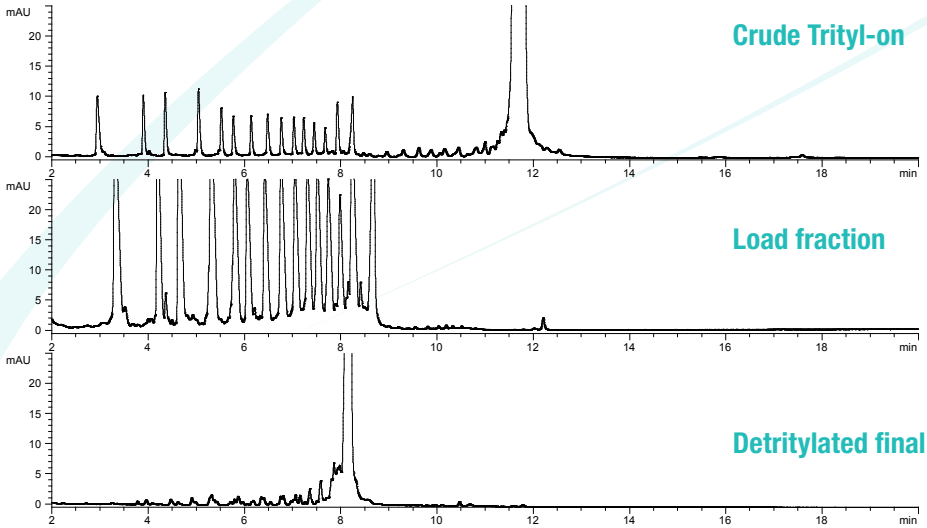
NOTE: *for large-scale purification, please refer to the QSP user's manual for appropriate deprotection reagent and purification solvent volumes or contact Phenomenex.*

RNA-TBDMS Purification Applications

Example 1

RNA: GGCUCCCCUCAACAACUUCTT (1 µmole)

Added 250 µL of deprotection cocktail to vial and heated @ 65 °C for 1.5 hr
Quenched with 1 mL of NH_4HCO_3



IEX Chromatography

OD_{260}

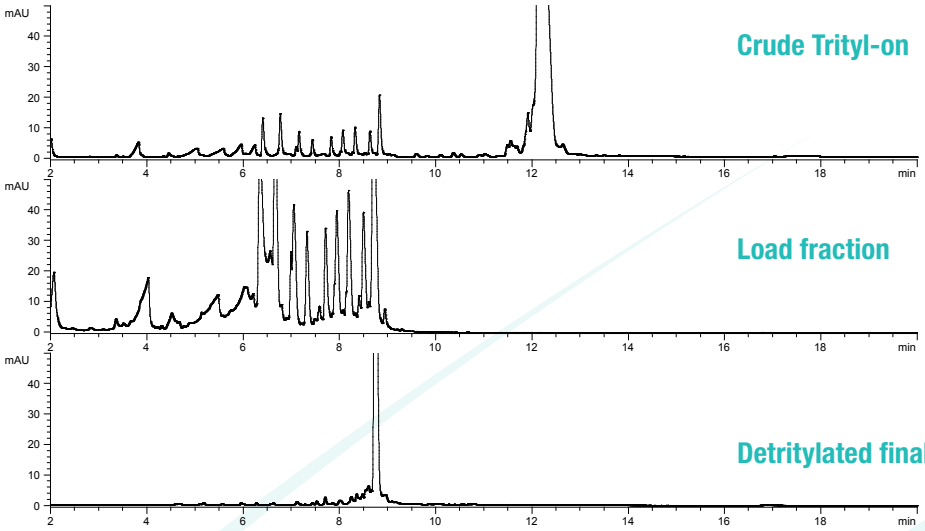
	Crude Trityl-on (1:100)	Load fraction	Detritylated final elution (1:100)	Recovery	Purity (Peak area)
Example 1	118.5	15.2	89	86 %	89 %

RNA-TBDMS Purification Applications (cont'd)

Example 2

RNA: 4181-3 GGAGAACCUGCCCAGCCGCTT (0.2 µmole)

2' deprotection: Added 100 µL of deprotection cocktail and heated @ 65 °C for 1.5 hr
Quenched with 400 µL of NH_4HCO_3



IEX Chromatography

OD_{260}

	Crude Trityl-on (1:100)	Load fraction	Detritylated final elution (1:100)	Recovery	Purity (Peak area)
Example 2	43.6	8.9	25.4	73 %	93 %

Support cleavage and primary deprotection:

- Add appropriate volume of a 1:1 mixture of 40 % methylamine and 33 % ethanolic methylamine to CPG column according to synthesis scale. Typically, 1 mL for 0.2 µmole, 1.5 mL for 1 µmole
- Incubate at 55 °C for 6 hours or overnight at room temperature
- Evaporate to dryness

Deprotection for 0.2 µmole Synthesis Scale

Reagents

- TBAF Tetrabutylammonium fluoride. Using either a 1 M TBAF solution in an aprotic solvent or prepare 15 % TBAF in same solvent
- Aprotic solvents: THF, DMF, NMP or DMSO
- 1 M Tris pH 7.4 (aqueous)

Protocol

- Add 100 µL of deprotection cocktail to dried RNA pellet and heat @ 50 °C for 10 minutes
- Heat @ 35 °C for 3 hrs
- Quench with 400 µL of 1 M Tris pH 7.4
- Total Volume 1 mL
- Proceed to QSP purification

QSP Purification for 0.2 µmole Synthesis Scale

Formats: 50 mg/ 96-Well Plate (Part No. 8E-S102-DGB)
50 mg/ 1 mL Cartridge (Part No. 8B-S102-DAK)

Purification Protocol:

- Add equal volume of Clarity QSP RNA-TOM loading buffer (500 µL) to RNA sample, total volume: 1-2 mL
- Condition: 1 mL MeOH (0.5 mL x 2)
- Equilibrate: 1 mL Water (0.5 mL x 2)
- Load Vol of RNA Oligo
- Detritylate: 1 mL 2 % DCA or TFA
- *Rinse: 1 mL Water (0.5 mL x 2)
- Dry sorbent @ 10" Hg (~1 min)
- *Elute: 1 mL 20 mM Na_2CO_3 / 50 % Methanol

Deprotection for 1.0 µmole Synthesis Scale

Reagents

- TBAF Tetrabutylammonium fluoride. Using either a 1 M TBAF solution in an aprotic solvent or prepare 15 % TBAF in same solvent
- Aprotic solvents: THF, DMF, NMP or DMSO
- 1 M Tris pH 7.4 (aqueous)

Protocol

- Add 250 µL of deprotection cocktail to dried RNA pellet and heat @ 50 °C for 10 minutes
- Heat @ 35 °C for 3 hrs
- Quench with 1 mL of 1 M Tris pH 7.4
- Total Volume 1.25 mL
- Proceed to QSP purification

QSP Purification for 1.0 µmole Synthesis Scale

Formats: 150 mg/ 3 mL Cartridge (Part No.: 8B-S102-SBJ)

Purification Protocol:

- Add equal volume of Clarity QSP RNA-TOM loading buffer (1.25 mL) to RNA sample, total volume: 2.5 mL
- Condition: 3 mL MeOH (1.5 mL x 2)
- Equilibrate: 3 mL Water (1.5 mL x 2)
- Load Vol of RNA Oligo
- Detritylate: 1.5 mL 2 % DCA or TFA
- *Rinse: 2 mL Water (1 mL x 2)
- Dry sorbent @ 10" Hg (~1 min)
- *Elute: 2 mL 20mM Na_2CO_3 / 50 % Methanol

*For users that avoid any salts in the final elution, a 1 mL rinse using a 20 mM concentration of an aqueous buffer (Na, NH_4 or tris) can be used in the rinse step. The RNA can then be eluted in a water/ methanol solution. To avoid potential depurination, it is recommended that the pH of the final solution be that of a physiological pH (7-8).

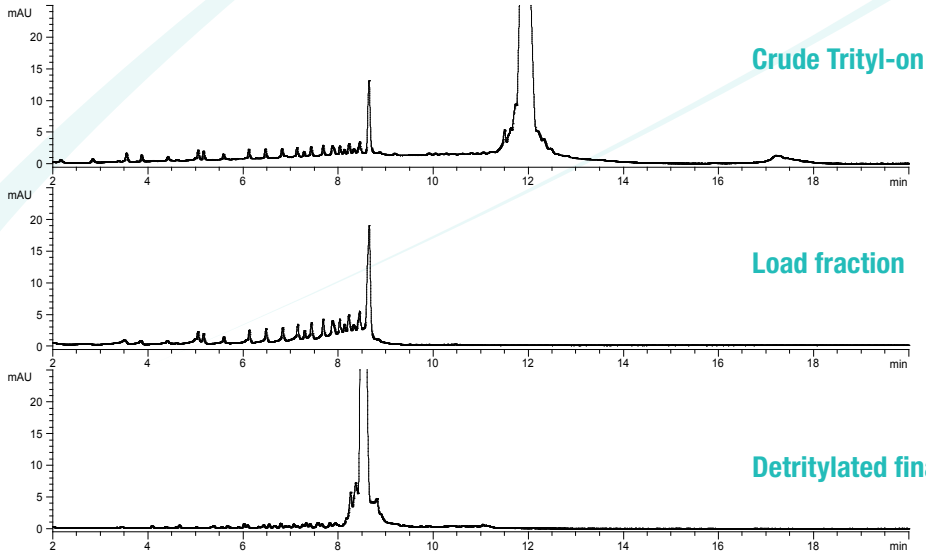
NOTE: *For large-scale purification users, please contact Phenomenex for appropriate deprotection reagents and purification solvent volumes.*

RNA-TOM Purification Applications

Example 3

RNA: GGA AAC CAC CGC UCU UUA ATT (0.2 µmole)

Added 100 µL of deprotection cocktail to dried RNA pellet and heated @ 35 °C for 3 hrs
Quenched with 400 µL of 1 M Tris pH 7.4



IEX Chromatography

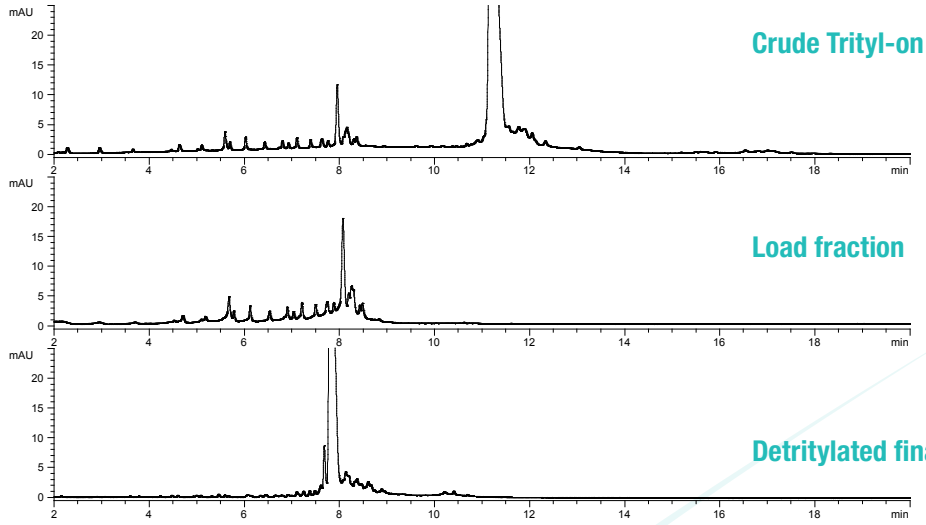
OD_{260}

	Crude Trityl-on (1:100)	Load fraction	Detritylated final elution (1:100)	Recovery	Purity (Peak area)
Example 3	26.8	5.5	15.8	74.2 %	89 %

Example 4

RNA: GAG UGA CCA CCU CAC UUG ATT (0.2 µmole)

Added 100 µL of deprotection cocktail to dried RNA pellet and heated @ 35 °C for 3 hrs
Quenched with 400 µL of 1 M Tris pH 7.4



IEX Chromatography

OD_{260}

	Crude Trityl-on (1:100)	Load fraction	Detritylated final elution (1:100)	Recovery	Purity (Peak area)
Example 4	18.5	5.1	10.9	81.4 %	92.6 %

Ordering Information

Formats

Part No.	Description		Unit
8E-S102-DGB	Clarity QSP	50 mg/ 96-Well Plate	1/Box
8B-S102-DAK	Clarity QSP	50 mg/ 1 mL Cartridge	50/Box
8B-S102-SBJ	Clarity QSP	150 mg/ 3 mL Cartridge	50/Box
8B-S042-LFF	Clarity QSP	5 g/60 mL Cartridge	16/Box

Buffer

Part No.	Description		Unit
AL0-8281	Clarity QSP RNA-TBMS Loading Buffer	100 mL	Ea
AL0-8282	Clarity QSP RNA-TBMS Loading Buffer	1 L	Ea
AH0-7858	Clarity Nuclease Free Water	1 L	Ea

NOTE: *Please contact Phenomenex for Clarity QSP RNA-TOM loading buffer ordering information.*