

General procedure:

1. Add 250 μ L TEA.3HF de-silylation solution to the dried oligoribonucleotide. Shake to dissolve the oligoribonucleotide and place in an oven at 65°C for 2.5 hours.
2. Remove from the oven and allow to cool in a refrigerator for a few minutes.
3. Add 1.75 mL TOP-RNA Quenching Buffer (PN: 75739025) to the de-silylation solution to quench.
4. Fit a TOP-RNA cartridge (PN: 7573915C, Tubes, TOP-RNA 100 mg 96/pk) into a needle tip previously fitted to a Varian Vac Elut 20 Extraction Manifold. Turn the vacuum on and adjust the pressure 7.0 inHg using the vacuum control valve. The vacuum should not need to be adjusted during the purification.
5. Add 0.5 mL acetonitrile to the cartridge to wet the medium.
6. As soon as possible, add 1 mL 2M TEAA to the cartridge to condition the medium.

(Short delays between the additions of the following solutions to the cartridge will not effect the purification.)

7. Add 1 mL aliquots oligoribonucleotide solution to the cartridge, using either a micropipette or a plastic pipette, if the sample vial is graduated.
8. Add 1 mL 10:90 (v/v) acetonitrile/2M TEAA to the cartridge.
9. Add 1 mL water to the cartridge.
10. Add 1 mL 2.0% TFA solution to the cartridge. Repeat with a further 1 mL 2.0% TFA solution.
11. Add 1 mL water to the cartridge. Repeat with a further 1 mL water.
12. Remove the cover, using the vacuum release valve. Place a glass test tube in the correct position in the rack and replace the cover. Add 1 mL 1M ammonium bicarbonate, 30% acetonitrile to the cartridge.
13. Turn off the vacuum and allow the pressure to equalise. Remove the cover and transfer the oligoribonucleotide solution to a suitable glass vial for storage with a pipette.

The purification should take approximately 10-15 minutes if carried out without any breaks between the additions of the solutions to the cartridge.

C) De-Silylation of Oligoribonucleotides

Reagents: *1-Methyl-2-pyrrolidinone, anhydrous, 99.5% (NMP)* *(Aldrich 32,863-4)*
 Triethylamine, puriss. p.a. ≥ 99.5%(GC) (TEA) *(Fluka 90340)*
 Triethylamine trihydrofluoride, 98% (TEA.3HF) *(Aldrich 34,464-8)*

De-silylation solution: *1.5 mL NMP*
 0.75 mL TEA
 1 mL TEA.3HF

The de-silylation solution should be made immediately before use in a 5 mL plastic vial. The components should be mixed in the order listed. The vial should be sealed and placed in an oven at 65°C for 5-10 minutes before use to dissolve any gel. The solution should be mixed and examined for gel particles on removal from the oven.

Add 250 µL of the de-silylation solution to the dried oligoribonucleotide in the cryo-vial. Shake the vial to dissolve the oligoribonucleotide. Place the vial in an oven at 65°C for 2.5 hours. Briefly remove the vial from the oven after approximately 10 minutes to mix the contents and confirm that the oligoribonucleotide has dissolved.

D) De-Silylation Quenching:

Reagents: *Quenching Buffer*

Quenching of the de-silylation solution should be carried out just prior to cartridge purification. Add 1.75 mL Quenching Buffer to the vial. Seal the vial and mix its contents by shaking.

E) Cartridge Purification

Reagents: *Acetonitrile, Far UV, HPLC Gradient Grade* *(Fisher Scientific A/0636/17)*
 2M Triethylammonium acetate, pH 7.0 (TEAA)
 0.5% (v/w) Trifluoroacetic acid
 1M Ammonium bicarbonate, 30% acetonitrile

2M Triethylammonium acetate, pH 7.0

Reagents: *Triethylamine, puriss. p.a. ≥ 99.5%(GC) (TEA)* *(Fluka 90340)*
 Acetic Acid, Ultra, ≥ 99.5% (GC/T) *(Fluka 45726)*

Weigh out 698.0 g de-ionised water into a 1 L Nalgene bottle. Add 100.0 g acetic acid to the bottle and mix it with the water by shaking. Add 202.0 g TEA to the bottle and mix it with the acetic acid solution by shaking (care: will generate heat). A small volume of TEA will be immiscible with the solution. Add a magnetic follower to the bottle and stir using a magnetic stirrer. Measure the pH of the solution with a

calibrated pH meter and adjust its pH to 7.0 with acetic acid. No separate TEA phase should be visible after neutralisation. The pH may be corrected with a few drops of TEA if necessary.

10% Acetonitrile, 90% 2M Triethylammonium acetate, pH 7.0

Measure out 10 mL acetonitrile in a 100 mL measuring cylinder and add it to a 125 mL Nalgene bottle. Measure out 90 mL 2M TEAA, pH 7.0 and add it to the acetonitrile in the bottle.

2.0% Trifluoroacetic acid

Reagents: *Trifluoroacetic acid, 99% (TFA)* (*Aldrich T6,220-0*)

Weigh out 98.00 g de-ionised water into a 125 mL Nalgene bottle. Add 2 mL TFA to the water, seal the bottle and mix by shaking.

1M Ammonium Bicarbonate, 30% Acetonitrile

Reagents: *Ammonium bicarbonate, Ultra, ≥ 99.5%(T)* (*Fluka 09830*)
 Acetonitrile, Far UV, HPLC Gradient Grade (*Fisher Scientific A/0636/17*)

Weigh out 5.53 g ammonium bicarbonate into a 125 mL Nalgene bottle. Measure out 70 mL de-ionised water in a 100 mL measuring cylinder and add it to the bottle. Dissolve the ammonium bicarbonate in the water. Measure out 30 mL acetonitrile in the measuring cylinder and add it to the bottle.

Cartridge Conditioning:

Fit a TOP-RNA cartridge into a needle tip previously fitted to a Varian Vac Elut 20 Extraction Manifold. Turn the vacuum on and adjust the pressure 7.0 inHg using the vacuum control valve. The vacuum should not need to be adjusted during the purification. Add 0.5 mL acetonitrile to the cartridge and allow the acetonitrile to be drawn through the cartridge under vacuum.

As soon as possible, add 1 mL 2M TEAA to the cartridge. The acetonitrile must not be allowed to evaporate completely before the 2M TEAA is added to the cartridge, if it does the 2M TEAA will not wet the medium and the purification will fail to work. Allow the 2M TEAA to flow through the cartridge under vacuum. Short delays between the additions of the following solutions to the cartridge will not effect the purification.

Sample Loading:

Add the oligoribonucleotide solution to the cartridge in 2 x 1 mL aliquots, using either a micropipette or a plastic pipette, if the sample vial is graduated. Allow the solution to flow through the tube under vacuum.

Elution of Trityl-Off Oligoribonucleotide Impurities:

Add 1 mL 10:90 (v/v) acetonitrile/2M TEAA to the cartridge and allow to flow through the cartridge as above.

Add 1 mL water to the cartridge and allow to flow through the cartridge as above.

De-tritylation of Oligoribonucleotide:

Add 1 mL 2.0% TFA solution to the cartridge and allow to flow through the cartridge as above. Repeat with a further 1 mL 2.0% TFA solution.

Add 1 mL water to the cartridge and allow to flow through the cartridge as above. Repeat with a further 1 mL water.

Elution of Pure Oligoribonucleotide:

Remove the cover, using the vacuum release valve. Place a glass test tube in the correct position in the rack and replace the cover.

Add 1 mL 1M ammonium bicarbonate, 30% acetonitrile to the cartridge and allow to flow through the cartridge as above.

Turn off the vacuum and allow the pressure to equalise. Remove the cover and transfer the oligoribonucleotide solution to a suitable glass vial for storage with a pipette.

The purification should take approximately 10 minutes if carried out without any breaks between the additions of the solutions to the cartridge.