

REMOVAL OF 2'-PROTECTING GROUPS (TOM or TBDMS) FROM RNA OLIGOS

Removal of 2'-Protecting Groups

Note: To minimize RNase degradation of the oligoribonucleotide, wear gloves and use sterile materials during the steps below.

Note: Use a fresh solution of tetrabutylammonium fluoride less than 6 months old, preferably stored under argon at room temperature.

1. Add 1ml of TBAF (Tetrabutylammonium fluoride solution 1 M in THF, Aldrich 21,614-3) to the dried oligo and vortex. (*Note:* TOM-protected oligos seem to be less soluble than TBDMS-protected oligos and it is imperative that they are fully dissolved for the desilylation reaction to proceed to completion. TOM-protected oligos should be warmed to 50°C with shaking for 10 minutes to dissolve.)
2. Incubate the solution > 6 hours at room temperature. (*Note:* For TOM-protected oligos, allow to cool to 35°C and continue to shake for > 6 hours.) Leaving the solution overnight at this point is not a problem.
3. Quench the reaction by adding 1 ml of 1M Tris buffer (pH 7.4) to the vial and shake well. This step also removes the 2'-hemiacetals.
4. Continue with one of the following desalting options.

Note: Attempts to precipitate RNA with butanol from a TBAF solution may not be effective and is not recommended.

Desalting the Oligoribonucleotide

Note: Desalting the deprotected RNA is critical especially if TOM protecting groups are present and gel purification will be used.

Option I: Poly-Pak II cartridge (Glen Research cat# 60-3100-01)

1. Since THF will affect binding, it is necessary to Speed-Vac the oligo to about 1/2 the original volume to remove the THF.
2. Add 1 ml of 0.1M TEAA (aqueous dilution of 2 M Aqueous Triethylammonium acetate, pH ~7, Glen Research cat# 60-4110-52) so that the final volume is about 2 ml. The loading volume of the sample onto the cartridge is not critical.

PolyPak protocol courtesy of Glen Research (www.glenres.com)

Procedure

The flow rate of solvents through the cartridge should be regulated at a rate of ~1-2 drops/second

Cartridge Preparation

1. Flush the cartridge with 4 ml of acetonitrile
2. Flush the cartridge with 4 ml 2M TEAA.

Desalting Procedure

3. Load the solution containing the oligo onto the cartridge
4. Flush the cartridge with 6 ml of 0.1M TEAA
5. Elute the desalted oligo by flushing the cartridge with 1 ml of 50% acetonitrile/water
6. Determine the A₂₆₀ units. Store any unused oligo as a lyophilized solid at -20°C.
7. The desalted oligo is now ready for purification using gel or HPLC techniques.

What's Happening

A good steady flow rate is usually sufficient to flush the bed of purification matrix.

Cartridge Preparation

1. The acetonitrile wets the resin and washes away any organic residues.
2. The TEAA acts as an ion pairing reagent to enhance the binding of the oligo to the resin.

Desalting Procedure

3. Save all flow-throughs and washes until the purified product is quantified!
4. The 0.1 M TEAA removes the salts from the cartridge.
5. The product should elute totally in 1 ml of 50% acetonitrile/water
6. Up to 150 A₂₆₀ units can be desalted on Poly-Pak II cartridges.

Option II: G-25 Sephadex

Sephadex is a size-exclusion media that allows small impurities to travel a longer mean flow path and to elute later than the relatively large RNA molecules, which elute first by exclusion from the internal pores of the media.

1. Using a vacuum centrifuge, concentrate the quenched solution from the previous section (Removal of 2'-Protecting Groups, step 3) to one-half its original volume (approximate).
2. Swell the G-25 Sephadex (Aldrich P/N 27,109-8) in deionized water for 4-5 hours. Then load a Bio-Rad Econo-column (0.7 x 20 cm, Bio-Rad P/N 737-721) with this slurry. A maximum of 100 ODU of crude RNA can be loaded on this column.
3. Allow the slurry to flow through the column until the Sephadex has settled (up to 16 cm).
4. Carefully load the RNA sample in a minimum amount of deionized water. After the sample has descended to the Sephadex level, carefully add additional deionized water to the top of the column (~10 mL).
5. After the sample has been loaded, collect 10x 1-mL fractions in sterile tubes. The RNA oligonucleotide generally elutes in tubes 2-5.
6. Assay each fraction on a UV spectrophotometer at 260 nm to determine which tubes contain the RNA oligonucleotide.
7. Pool the fractions containing the RNA, evaporate to dryness.
8. The desalted oligo is now ready for purification using gel or HPLC techniques.

Option III: OPC™, (Oligo Purification Cartridge - Applied Biosystems cat # 400771)

An oligonucleotide purification cartridge can also be used for desalting. OPC desalting is a hydrophobicity-based separation. The desalting procedure has only a limited capacity of approximately 10-20 ODU per OPC (for oligoribonucleotides synthesized Trityl-Off).

1. Using a vacuum centrifuge, concentrate the quenched solution from the previous section (Removal of 2'-Protecting Groups, step 3) to one-half its original volume (approximate).
2. Wash the OPC with 5 mL of acetonitrile, and then with 5 mL of 2 M TEAA.
3. Dissolve the viscous semi-solid (from step 1 above) in 1 mL of 0.1 M TEAA, then load it onto the OPC, passing it through the cartridge twice at 1-2 drops per second. Save the eluate because occasionally the oligoribonucleotide may fail to adhere completely to the OPC.
4. Wash the OPC with 5 mL of 0.1 M TEAA, followed by 10 mL of deionized water. Then elute the desalted oligoribonucleotide with 1 mL of 50% acetonitrile.
5. Quantitate the oligo using a UV spectrophotometer and reading the absorbance at 260 nm.
6. The desalted oligo is now ready for purification using gel or HPLC techniques.

Sephadex and OPC protocols courtesy of Applied Biosystems (www.appliedbiosystems.com)