Applied Biosystems

Oligonucleotide Purification Cartridges

400771 DNA Synthesis Product

Qty: 10 **NOTICE: For R & D Use Only**

Lot: A1L088

Patent Pending

Solutions needed:

- HPLC grade acetonitrile, 5 mL (Part No. 400262)
- 2.0 M triethylamine acetate, 5 mL (Part No. 400613)
- Deionized water
- 1.5M ammonium hydroxide, 15 mL (1:10 dilution of concentrated ammonium hydroxide in deionized water)
- 2% trifluoroacetic acid, 5 mL (1:50 dilution of Neat TFA Part No. 400137)
- 20% v/v acetonitrile in deionized water, 1 mL

1. After completion of trityl-on synthesis, cleave the oligonucleotide from the support and deprotect following normal protocols for the synthesis method utilized.
2. Connect an all polypropylene syringe (Aldrich Z11686-6); an OPC™ cartridge, and male-to-male luer tip. Make sure all fittings are snug. The OPC™ cartridge may be immobilized with a laboratory clamp.
3. Flush the cartridge with 5 mL HPLC grade acetonitrile, followed by 5 mL 2.0M triethylamine acetate. Remove the syringe from the OPC™ cartridge before removing the plunger; then re-insert the syringe barrel prior to the next addition.
4. Dilute an aliquot containing ~ 20 OD units of the crude, deprotected oligonucleotide still in concentrated ammonia with one third volume of deionized water. The final volume of the solution should be 1 to 4 mL.

   Important: Keep the flow rate at 1 to 2 drops per second for all subsequent reagent additions.
5. Place this solution (step 4) in the syringe and slowly push it through the cartridge. Save the eluted fraction, place it in the syringe, and gently push it through the cartridge. Again, this will load 1 to 5 OD units of the crude oligonucleotide (depending on length, sequence, and synthesis quality) onto the cartridge.
6. Slowly wash the cartridge with 3 × 5 mL 1.5M ammonium hydroxide.
7. Flush the cartridge with 2 × 5 mL deionized water.
8. Detritylate the OPC™ bound oligonucleotide with 5 mL of the 2% trifluoroacetic acid solution. Gently push ~ 1 mL through the cartridge, wait 5 minutes, then flush the remaining TFA solution through the cartridge.
9. Flush the cartridge with 2 × 5 mL deionized water.

   For sequences ≥ 40 bases, add this step:

   9a. Gently push through the cartridge 1 × 5 mL 1.5M ammonium hydroxide, followed by 2 × 5 mL deionized water.
10. Elute the purified, detritylated oligonucleotide by slowly washing the cartridge with 1 mL of the 20% acetonitrile solution.
11. Determine the OD units at 260 nm with an aliquot of the eluate from step 10.
12. Store the OPC™ purified oligonucleotide as a dry solid at – 20 °C.

Helpful hints:

- Store the remaining crude solution in ammonia. This will not harm the product in any way and will save you time.
- Use fresh ammonia for cleavage on the instrument and for deprotection at 55 °C to acquire optimum separation.
- Store the TEA-Ac and 15M ammonium hydroxide at 4 °C. Make the 1.5M solution of ammonium hydroxide daily, as needed.
- Remove the syringe from the OPC™ cartridge prior to removing the plunger from the syringe.

Note:

- Don't be concerned that TFA will harm your sample. Once deprotected, the bases are 10 times less susceptible to depurination.
- The 260 nm/280 nm ratio of purified synthetic DNA is highly sequence dependent and may differ from the typical 1.8 value associated with genomic DNA isolated from a natural source.