

Cleavage and Deprotection of Halogenated Trityl-On DNA Oligos

Materials:

- 5 ml Luer-slip syringes (Becton Dickinson #301028) - supplied with oligos
- 1 dram glass vials (Wheaton #224702) and green Teflon-lined caps (All-Pak #5200) - supplied with oligos
- ethanolic ammonium hydroxide, prepared by mixing 1 volume of 100% ethanol with 3 volumes of concentrated (28-30%) ammonium hydroxide (Baker #9721-01)

If oligos are to be dried:

- Triethylamine (Aldrich #T-0886)
- 0.05M Triethylammonium bicarbonate, prepared by dilution from a 1M stock (Aldrich #T-7408)

Note: It is critical to use fresh ammonium hydroxide to ensure complete cleavage and deprotection. We recommend using small 500ml bottles stored tightly capped at -20° and discarded if they have not been used up within a month.

Note: Since halogenated oligos are somewhat light-sensitive, minimize exposure of the oligos to light when performing the cleavage and deprotection.

EtOH Wash

Using a 5cc syringe, gently pass 5ml of 100% EtOH through the column at a rate of about 1 drop/sec. This removes residual synthesis reagents that may lead to cleavage of the halogenated base during the deprotection reaction.

Cleavage

1. Draw 3mls of ethanolic ammonium hydroxide up into a 5cc Luer-slip syringe; remove any trapped air.
2. Firmly attach the syringe to the bottom of the synthesis column.
3. Secure a second 5cc syringe to the top of the column, pushing the syringe bodies (not the plungers!) together firmly to ensure that they will not fall apart later.
4. Slowly push approximately 500 uL of ethanolic ammonium hydroxide from the lower syringe into the upper syringe. It may be helpful to gently pull up the plunger on the top syringe while pushing the ethanolic ammonium hydroxide from the bottom syringe.
5. Allow the column to incubate in the dark with the ethanolic ammonium hydroxide for 30 minutes at room temperature. (It may be helpful to place the syringe-column assembly in a 1000ml beaker to keep it upright during the incubation).
6. Repeat steps 4 and 5 three more times until the column has incubated for 2 hours in the ethanolic ammonium hydroxide.
7. Push the remainder of the ethanolic ammonium hydroxide from the lower syringe into the upper syringe.

8. Invert the assembly, and manipulate the syringes so as to get all of the liquid into what is now the lower syringe (if necessary, you can add a little air by fully depressing the top plunger, removing the top syringe while gently pulling down on the lower plunger, and then replacing the top syringe). These contortions are necessary because ethanolic ammonium hydroxide has a tendency to drip out of the syringe if the tip is down.

9. While keeping the lower syringe pointed upwards, remove the top syringe and the column. Invert a glass vial over the tip of the lower syringe, and smoothly invert both together. Push down on the syringe plunger to dispense the ethanolic ammonium hydroxide solution into the vial. Cap the vial tightly using the green, teflon-lined cap. (Avoid over-tightening the cap since this may cause the neck of the vial to break - note that the caps have crushable foam under the teflon and hence are not re-usable).

10. With a Sharpie marker, draw a line on the vial to indicate the bottom of the meniscus. This will serve as an indicator to assure that the vial remained sealed during the deprotection.

Deprotection

1. Incubate the oligo for 4 hours at 55°C keeping the oligo protected from light.

2. Following the deprotection, chill the vial for 10 minutes at -20°C. (Caution: Do not attempt to open the heated vial until it has been thoroughly cooled!!).

3. After chilling, check the ammonia level to verify that the vial remained sealed and that the ethanolic ammonium hydroxide concentration remained constant. If the level is significantly below the mark, the oligo should be transferred to a fresh vial, dried, resuspended in ethanolic ammonium hydroxide and re-protected (use a fresh cap!) to ensure complete removal of the base-protecting groups.

At this point we recommend putting the oligo directly on HPLC, probably diluting it to reduce the ethanolic ammonium hydroxide concentration. If you wish to dry it using the protocol we used previously, that procedure is described below:

Previous Drying Procedure

1. Open the vial and incubate for 1 hour at 37°C in a fume hood shielding the vial from direct light. This step partially removes the ethanol from the mix and decrease the risk of boiling when placed in the speed-vac.

2. Cap the vial and chill at -20°C for 20 minutes.

3. Dry in a speed-vac without heat and in darkness adding several drops of triethylamine (TEA) every 45 minutes until the oligo is completely dry (the glass vials fit directly into our rotors, but you may need to transfer to something that will fit into your rotor). The addition of TEA is intended to keep the solution alkaline to prevent loss of the trityl group.

4. When dry, add 1ml of 0.05M triethylammonium bicarbonate (TEAB) and 50uL of TEA. The oligo should be stable for at least several weeks if stored at -20°C.