

Cleavage and Deprotection of Unmodified 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
- 500ml bottles 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.

Cleavage

1. Draw 3ml of 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 500uL of 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 ammonium hydroxide from the bottom syringe.
5. Allow the column to incubate with the ammonium hydroxide for 15 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 1 hour in ammonium hydroxide.
7. Push the remainder of the ammonium hydroxide from the lower syringe into the upper syringe.
8. Invert the assembly and manipulate the plungers so as to move 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 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, then smoothly invert both together. Push down on the syringe plunger to dispense the 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. If the label on the vial states "Fast G", incubate the sealed vial for 1 hour at 65°C. If the "Fast G" label is not present, incubate for 12 hours at 55°C.
2. Following the deprotection, chill the vial for 30 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 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 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 ammonium hydroxide concentration. If you wish to dry it using the protocol we used previously, that procedure is described below:

Previous Drying Procedure

1. Dry in a speed-vac without heat 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.
2. 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.