

Cleavage and Deprotection of 3' ddC Trityl-On DNA Oligos

Materials:

- 1 dram glass vials (Wheaton #224702) and green Teflon-lined caps (All-Pak #5200) are supplied with oligos
- 500ml bottle 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 after opening.

Cleavage and Deprotection

Note: When opening the synthesis column, place a large piece of weigh paper under the column in order to collect any of the solid support that may spill.

1. Unscrew the black retaining ring from the top of the synthesis column.
2. While holding the base of the column firmly on the lab bench, carefully unsnap the top luer fitting from the column body. The solid support will remain in the column body.
3. Pour the solid support from the column body into the glass vial. Tap to ensure that all of the support has been removed from the column body.
4. Add 3 mls of concentrated ammonium hydroxide to the glass vial and 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).
5. With a Sharpie marker, draw a line on the vial at the bottom of the meniscus. This will serve as an indicator to assure that the vial remained sealed during the deprotection.
6. Incubate the sealed vial for >12 hours at 55°C.

7. Chill the vial for 30 minutes at -20°C . (Caution: Do not open the vial until it has been thoroughly cooled!!).

8. Check the ammonia level to verify that the vial remained sealed so that the ammonium hydroxide concentration did not decrease. If the level dropped significantly below the mark, the oligo should be transferred to a fresh vial, dried using the protocol described below, resuspended in ammonium hydroxide and re-protected (use a fresh cap!) to ensure complete removal of the base-protecting groups.

9. Briefly vortex the vial to release any oligo that may be trapped within the solid support and allow the solid support to settle (usually 1 to 2 minutes).

10. Transfer the supernatant to a suitable tube for drying or diluting and discard the solid support.

At this point we recommend putting the oligo directly onto HPLC without drying it, diluting it to reduce the ammonium hydroxide concentration if necessary. If you wish to dry it, the protocol we previously used is described below:

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 a vial 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 .