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IMPROVED DEPROTECTION WITH SODIUM HYDROXIDE AND DMT-ON PURIFICATION

Deprotection with Sodium Hydroxide

Typically, 0.4 M NaOH in MeOH/water 4:1 (v/v) for 17 hours at room temperature is used to deprotect oligos that are sensitive to deprotection with amine containing bases like ammonium hydroxide and AMA.

Use of dmf-dG

Quite surprisingly, dimethylformamidine (dmf), a popular protecting group for guanosine, is remarkably resistant to the sodium hydroxide solution. While an isobutyryl-protected dG is cleanly deprotected in 17 hours, the dmf group requires over 72 hours at room temperature.

Use of Ac-dC

In addition, if benzoyl-protected dC is used, nucleophilic displacement of benzamide can occur due to hydroxide attack of the C4 carbon, leading to deamination and a dC to dU mutation. This can be avoided if acetyl-protected dC (Ac-dC) is used during synthesis.

Use of NaOH

A final concern is that the yield of oligos can be highly variable when using 0.4 M NaOH in MeOH/water for deprotection. The CPG will tend to fuse to a solid cake in the bottom of the vial of larger-scale syntheses and the oligo will tend to precipitate onto the CPG. However, we found that by first briefly sonicating the vial to break up the CPG, pipetting off the supernatant and then rinsing the CPG with water, will consistently give good oligo yields.

0.4 M NaOH has to be neutralized or desalted prior to drying down. For neutralization, add 28 µL of glacial acetic acid per mL of 0.4 M NaOH. Neutralization is NOT compatible with DMT-ON purification.

Note

The use of sodium hydroxide necessitates a desalting step. This can be accomplished, while simultaneously purifying the oligo, by using a Glen-Pak™ purification cartridge. An optimized protocol (for a 1 µmole synthesis scale or smaller) is given below.

Protocol for Deprotection and DMT-On Glen-Pak™ Purification

1. Make a fresh solution of 0.4 M NaOH in MeOH/water 4:1 (v/v).
2. If it is desired to eliminate the cyanoethyl protecting groups on the phosphate backbone, treat the column with 3 mL of 10% diethylamine (DEA) in ACN for 2 minutes, pushing the solution back and forth occasionally. Rinse with ACN and air dry the CPG.
3. Transfer the CPG to a vial and add 1 mL of 0.4 M NaOH in MeOH/water 4:1 (v/v).
4. Allow to react for 17 hours at room temperature.
5. Briefly sonicate the vial to break up the CPG.
6. Pipette off the supernatant and transfer to a clean vial. Rinse the CPG with 250 µL of water and combine with the cleaved oligo.
7. Dilute to 10 mL with 100 mg/mL NaCl in water.
8. Load onto a prepped Glen-Pak DNA cartridge (60-5200-xx) fitted with a 10 mL syringe and purify using the standard protocol but beginning with the Salt Wash Step – i.e., the 2 mL rinse with 100 mg/mL NaCl containing 5% ACN.
9. Dry the purified oligo.

http://www.glenresearch.com/Technical/GlenPak_UserGuide.pdf