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GENERAL PROCEDURE FOR LABELLING OF THIOL-MODIFIED OLIGONUCLEOTIDES

This general procedure can be used to conjugate thiol-modified oligonucleotides with thiol specific (e.g., iodoacetamide, maleimide) derivatives of fluorescent dyes which are not suitable for use as cyanoethyl phosphoramidites.

PROCEDURE

Step 1a: Oligo Sulfhydryl activation (Trityl-protected oligo)

Dissolve trityl-protected oligo (15-25 A_{260} units or 0.2 μ mole synthesis) in 0.1M TEAA (100 A_{260} units/mL). Add 0.15 volume 1M $AgNO_3$, vortex to mix. Let stand for 30 min. Add 0.20 volumes 1M DTT, vortex to mix. Let stand for 20 min.

Step 1b: Oligo Sulfhydryl activation (Oligo-disulfide)

Dissolve oligo disulfide (15-25 A_{260} units) in 0.25mL 100mM DTT, pH 8.3 - 8.5. Incubate at room temperature for 30 minutes.

Step 2: Removal of DTT and other reaction byproducts and change to conjugation buffer

Load entire sample on a NAP-10 (or equivalent) column equilibrated with 50mM sodium phosphate, pH 6.0. Allow to drip through. Add 0.75mL of 50mM sodium phosphate, pH 6.0 and allow to drip through. Elute with 1mL sodium phosphate pH 6.0. Collect for conjugation.

Step 3: Conjugation

Dissolve thiol reactive ligand in sodium phosphate, pH 6.0 (\approx 20mM final concentration). If thiol reactive ligand is not soluble in water dissolve in appropriate solvent (DMF or DMSO) at the same concentration. Add 0.2mL thiol reactive ligand to activated, desalted thiol modified oligo from step 2. Vortex and incubate at RT for 2-4 hours or overnight at 4°C.

Step 4: Purification

Purify oligo conjugate by PAGE, RP HPLC or ion exchange HPLC.

