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GENERAL PROCEDURE FOR LABELLING OF AMINO-MODIFIED OLIGONUCLEOTIDES

This general procedure can be used to conjugate amino-modified oligonucleotides with active ester or isothiocyanate derivatives of fluorescent dyes which are not suitable for use as cyanoethyl phosphoramidites. At pH 9, conjugation occurs virtually exclusively at the amino group and does not react with the exocyclic amino groups of the nucleosides.

Note that oligos deprotected in AMA may require a desalting step prior to conjugation to prevent methylamine from reacting with the NHS ester.

1. Dissolve the product from a 0.2 µmole synthesis of amino- modified oligonucleotide (i.e., approximately 0.1 - 0.2 µmoles of free primary amines) in 0.7mL of sterile distilled water.
2. Add 0.1mL of 10X conjugation buffer (1M $NaHCO_3/Na_2CO_3$, pH9).
3. Freshly prepare a 10mg/mL solution of active ester in DMF. Add 0.2mL of the solution to the reaction mixture.
4. Vortex and allow the mixture to stand at least 2 hours. (Note: Overnight reaction may be more convenient.)
5. Desalt the reaction mixture on a reverse phase cartridge or a Glen Gel-Pak™ desalting column to remove the excess label. Purify the product using RP HPLC if necessary.