Labeling of Amino-Modified Oligonucleotides with Dye NHS esters

Reaction Setup: Prepare a stock solution of 1 mg of the NHS-activated dye in 50 µL of anhydrous dimethyl formamide (DMF) or dimethyl sulfoxide (DMSO). 20 µL of this dye stock solution are added to a solution containing 20 - 30 nmol of the amino-modified oligonucleotide dissolved in 200 µL of a 50–100 mM bicarbonate buffer (pH 7.5 - 8.0). The mixture is allowed to stir for an additional 1–3 h at 25 ºC.

Notes: 1). In most cases the labeling reaction is completed within 30 minutes. 2). It is critical that the solution used for labeling should be free of amines (TRIS buffer is not suitable as a labeling buffer for dye NHS-esters). Oligonucleotides stored in buffers containing amines need be dialyzed against the labeling buffer (phosphate-buffered saline (PBS), or sodium bicarbonate). In some instances it might be necessary to purify the oligonucleotide before labeling. 3). Amino-modified oligo purification: 100 µg of the oligonucleotide are dissolved in 100 µl doubly distilled water and the solution is extracted three-times with chloroform and thereafter the oligonucleotide is precipitated by adding 20 µl of a 3 M sodium chloride solution and 250 µl of ethanol. The solution is mixed well and cooled for at least 30 min. at –20 °C. After centrifugation for 20 - 30 min at 13,000 g, the supernatant is separated and discarded. The pellet is washed twice with cold ethanol (70%), dried (not entirely dry, otherwise it might be difficult to redissolve) and resolubilized in 150 - 250 µl Tris/HCl buffer, pH 8.0 or in distilled water.

Purification of the Dye-Oligonucleotide Conjugate: The labeled oligonucleotide is first purified by repetitive ethanol precipitation as described above (see Note 3). The final purification is done by HPLC-separation on a reversed phase (C-18) column using an acetonitrile/H2O (80:20, v/v) gradient. Load the solution onto the column and run a linear solvent gradient of 0 - 75 % acetonitrile in water.

Note: Purification of the labeled oligonucleotide may be achieved using commercially available quick separation columns following the instructions given by the supplier.

Storage of the Dye-Oligonucleotide Conjugates: Dye-conjugates are to be stored under similar conditions as unlabeled oligonucleotids.

References:

Oligonucletide Labeling Reagents, AAT Bioquest (2014).