SETA BioMedicals

Fluorescent Tools for BioMedical Applications

Labeling of Amino-Modified Oligonucleotides with NHS esters

Prepare a solution of 75 nmol of the NHS-activated dye in 50 µL of anhydrous dimethyl formamide (DMF) or dimethyl sulfoxide (DMSO and add it to a solution containing 25 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. However, in most cases the labeling reaction will be completed within 10 - 20 minutes.

It is important to note that the solution used for labeling should be free of amines and TRIS buffer is therefore not suitable as a labeling buffer for NHS-esters. Oligonucleotides stored in buffers containing amines are to 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.

Purification of the Amine-Modified Oligonucleotide

For 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 13,000g, 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

First the labeled oligonucleotides are purified by repetitive ethanol precipitation as described above. Final purification is done by HPLC-separation on a reversed phase (C-18) column using an acetonitrile/H_2O (80:20, v/v) gradient. Load the solution onto the column and run a linear solvent gradient of 0 - 75% acetonitrile in water. 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 oligonucleotides.