## General Protein Labeling Procedures

A stock solution of 1 mg of the NHS-activated dye in 100 - 200 µL of anhydrous dimethyl formamide (DMF) or dimethyl sulfoxide (DMSO) is prepared. Then aliquots of 5, 10, 25, and 50 µL of this dye stock solution are added slowly to a solution of 5 mg IgG dissolved in 1 ml of a 50 - 100 mM bicarbonate buffer (pH 8.0 - 9.0). This mixture is allowed to stir for an additional 1 - 3 h at 25 °C. However, in most cases the labeling reaction will be completed within 5 - 10 minutes. To increase the degree of labeling a higher starting ratio of NHS-ester vs. protein should be used.

As the number of amino-groups in every protein is different it is important to adjust the D/P starting ratios in order to find the appropriate degree of labeling (DOL). The protein solution used for labeling should be free of amines and a TRIS buffer is therefore not suitable as a labeling buffer for NHS-esters. Antibodies stored in buffers containing amines are to be dialyzed against the labeling buffer (phosphate-buffered saline (PBS), or sodium bicarbonate).

### Purification of Dye-Protein Conjugates

Separation of the dye–protein conjugate from non-conjugated dye is achieved using gel permeation chromatography on a 1.5 x 25 cm column (stationary phase: Sephadex G-25; eluent: 67 mM PB, pH 7.4). The fraction with the shortest retention time containing the colored dye–protein conjugate is collected. This first colored band will be the desired conjugate. The second, slower moving band in general contains the unlabeled free dye (hydrolyzed NHS-ester).

### Determination of Dye-to-Protein Ratio (D/P)

This procedure including the x-factor values are provided in a separate technical note on our website.

### Storage of Dye-Protein Conjugates

Dye-protein conjugates are to be stored under similar conditions as used for the unlabeled protein. Typical storage temperatures are 4°C and sodium azide can be added to avoid bacterial growth. For long-term storage, prepare smaller aliquots and freeze. Avoid repeated freezing and thawing. Protect from light.