SETA BioMedicals

Fluorescent Tools for BioMedical Applications

General Procedure for Labeling of Proteins with Maleimides

Maleimides react with thiol groups in proteins or peptides or thiol-modified oligonucleotides in a Michael addition type reaction to form a covalent bond that is relative stable. The optimal pH for this reaction is between pH 6.8 - 7.5 and the most convenient way is to perform this procedure in phosphate buffered saline (PBS) solution. At this pH the primary amino groups in proteins are mostly protonated and show reduced reactivity towards maleimides.

Labeling Procedure

Dissolve the protein at 10mg/ml in PBS at pH 6.8 - 7.5. Prepare a 1 - 10 mM stock solution of the maleimide in anhydrous DMSO or DMF. It is important that the solutions are freshly prepared before each labeling procedure as they are not stable for an extended period of time. Depending on the desired D/P end ration add a 10 - 20 fold molar excess of reactive dye to the protein solution. Stir and incubate 2 hours at room temperature. To remove any excess of residual reactive dye, a small excess of glutathione or mercapto-ethanol can be added to the solution after the finalizing the labeling procedure. If the labeling yields are repeatedly low we strongly would recommend doing these reactions in an inert atmosphere to prevent oxidation of thiols.

Purification of the Dye-Conjugate

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 (unreacted maleimide).

Determination of the Dye-to-Protein ratio (D/P)

The 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.