Protocol for Labeling Biomolecules with 5-TAMRA NHS Ester

Overview:

This protocol provides a general method for labeling primary amine-containing biomolecules, such as proteins or peptides, with 5-carboxytetramethylrhodamine NHS ester (5-TAMRA-SE). NHS esters react efficiently with primary amines in aqueous buffers, forming stable amide bonds.

Materials:

- 5-TAMRA NHS ester (store desiccated at -20°C, protect from light)
- Target biomolecule (e.g., antibody, peptide)
- DMSO or DMF (anhydrous) for dye dissolution
- Sodium bicarbonate or phosphate buffer (0.1 M, pH 8.3)
- Optional: desalting column or dialysis tubing

Procedure:

1. Dissolve 5-TAMRA NHS ester in anhydrous DMSO or DMF to a concentration of 10 mg/mL (prepare fresh).
2. Dissolve the biomolecule in 0.1 M sodium bicarbonate or phosphate buffer (pH 8.3) to 1-10 mg/mL.
3. Add 5-TAMRA NHS ester solution dropwise to the biomolecule at a 5-10 molar excess.
4. Incubate the reaction at room temperature for 1 hour, protected from light.
5. Quench the reaction by adding 1 M Tris buffer (pH 7.5) to a final concentration of 50 mM (optional).
6. Purify the labeled biomolecule using gel filtration (e.g., Sephadex G-25), spin columns, or dialysis.

Notes:

- Avoid buffers containing primary amines (e.g., Tris or glycine) during labeling.
- Adjust dye-to-protein ratios based on application and desired labeling density.
- Store the labeled conjugate at 4°C protected from light.

Applications:

- Fluorescence microscopy

- Flow cytometry

- Fluorescence-based assays