Texas Red NHS Ester Labeling Protocol

Purpose

This protocol describes how to label primary amine-containing biomolecules such as antibodies, proteins, or oligonucleotides using Texas Red NHS Ester (single isomer). The NHS (N-hydroxysuccinimide) ester reacts efficiently with primary amines to form stable amide bonds.

Materials Required

- Texas Red NHS Ester (lyophilized)
- Anhydrous DMSO or DMF
- Target biomolecule (e.g., antibody, protein)
- Reaction buffer (e.g., 0.1 M sodium bicarbonate, pH 8.3)
- Optional: Desalting column or dialysis tubing for purification

Protocol

1. Reconstitute Texas Red NHS Ester in anhydrous DMSO to a concentration of 10 mg/mL.
2. Prepare your biomolecule in 0.1 M sodium bicarbonate buffer (pH 8.3) at 1-10 mg/mL.
3. Add the dye solution to the biomolecule solution at a molar ratio of 5-10:1 (dye:biomolecule).
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 20 mM.
6. Purify the labeled biomolecule using a desalting column, dialysis, or spin filter.
7. Store the labeled product at 4°C in the dark for short-term use, or at -20°C for long-term storage.

Notes

- Ensure all buffers are amine-free to prevent competition with the dye.
- Degree of labeling (DOL) can be determined spectrophotometrically using absorbance at ~595 nm.
- Avoid freeze-thaw cycles of the dye stock solution.