

Protocol: Labeling Biomolecules with 6-Carboxy-X-Rhodamine (6-ROX) NHS Ester

1. **Dissolve the Dye**

- Dissolve 1 mg of 6-ROX NHS ester in 100 μ L of anhydrous DMSO or DMF to prepare a 10 mg/mL (approx. 13 μ M) solution.

2. **Prepare the Biomolecule**

- Dissolve the amine-containing biomolecule (e.g., protein, antibody, or amine-modified oligo) in a pH 8.3 sodium borate buffer.

3. **Perform the Labeling Reaction**

- Add dye solution to the biomolecule in a molar ratio of 5–10:1 (dye:biomolecule).
- Incubate for 1 hour at room temperature in the dark with gentle mixing.

4. **Quench the Reaction (Optional)**

- Add 10 mM Tris-HCl (pH 7.5) to quench any unreacted NHS ester.
- Incubate for 10 minutes at room temperature.

5. **Purify the Labeled Biomolecule**

- Use desalting spin columns, dialysis, or HPLC to separate the labeled biomolecule from free dye.

6. **Storage**

- Store the labeled product in buffer at 4°C for short term or -20°C for long term, protected from light.

Notes:

- Ensure all buffers are free of primary amines (e.g., avoid Tris during labeling).
- Keep dye solutions and reactions protected from light to prevent photobleaching.
- Optimization may be required depending on the biomolecule and intended application.