

## FITC Amine Labeling Protocol

### Overview:

This protocol outlines the labeling of primary amine-containing biomolecules (e.g., antibodies, peptides, proteins) using fluorescein 5(6)-isothiocyanate (FITC). The isothiocyanate group reacts with primary amines to form a stable thiourea linkage.

### Materials:

- FITC (5(6)-isomer mix, emp Biotech)
- Target biomolecule (e.g., protein, antibody)
- DMSO or DMF (for dye dissolution)
- Sodium bicarbonate buffer (0.1 M, pH 9.0)
- Desalting column or dialysis membrane (optional)

### Procedure:

1. Dissolve FITC in dry DMSO to a concentration of 10 mg/mL. Prepare fresh.
2. Dissolve your protein or amine-containing biomolecule in 0.1 M sodium bicarbonate buffer (pH 9.0).
3. Add FITC to the biomolecule solution at a molar ratio of 5:1 (FITC:protein) for typical labeling.
4. Incubate in the dark at room temperature for 1-2 hours with gentle stirring.
5. Quench the reaction by adding 10 mM Tris buffer (optional).
6. Remove excess FITC using a desalting column, spin filter, or dialysis (depending on molecule size).
7. Store labeled product at 4°C protected from light.

### Notes:

- Avoid amine-containing buffers such as Tris during labeling.
- Use freshly prepared FITC solutions.
- Optimize dye:protein ratio for specific applications.

### Applications:

- Fluorescent detection of labeled antibodies in flow cytometry
- Fluorescence imaging of proteins or peptides in microscopy
- Bioconjugation workflows involving amine-tagged biomolecules