Number  Description
46424  FITC, 1g
46425  FITC, 100mg
          Chemical name: Fluorescein isothiocyanate
          Molecular weight: 389.38
          Extinction coefficient: 70,000 M⁻¹ cm⁻¹
          (in aqueous buffer, pH 9)
          Ex/Em wavelength: 494/518nm
          CAS # 3326-32-7

46112  TRITC, 10mg
          Chemical name: Tetramethylrhodamine-5- (and 6)-isothiocyanate
          Molecular weight: 478.97
          Extinction coefficient: 100,000 M⁻¹ cm⁻¹
          (at 544 nm in methanol)
          Ex/Em wavelength: 541/572nm
          CAS #6749-36-6

Storage: Upon receipt store at -20°C. Store product in the foil pouch with desiccant to protect from light and moisture. Product is shipped at ambient temperature.

Introduction
Thermo Scientific™ FITC and TRITC are among the most simple and commonly used reagents for protein fluorescent labeling. These isothiocyanates react to amino, sulphhydryl, imidazoyl, tyrosyl or carbonyl groups on proteins; however, only the derivatives of primary and secondary amines generally yield stable products. Reactions are most efficient at pH 8-9 and must be performed in an amine-free buffer such as carbonate/bicarbonate. Antibody and other proteins can be effectively labeled with several fluorophore tags per protein molecule when reacted with a 15- to 20-fold molar excess of isothiocyanate-activated fluorophore. Excess nonreacted and hydrolyzed reagent can be removed by dialysis, gel filtration or dye removal resin.
**Procedure for Antibody Labeling with FITC**

**Materials Required**
- Conjugation buffer: 50mM borate buffer, pH 8.5 (Product No. 28384)
- Antibody solution: dissolve ~1mg of antibody in 0.5mL of Conjugation Buffer
- Device to remove excess dye, such as a Dye Removal Columns (Product No. 22858), Thermo Scientific™ Zeba™ Spin Desalting Column (Product No. 89891) or Slide-A-Lyzer™ Dialysis Cassette, 10K MWCO (Product No. 66380)

**Procedure**
1. Dissolve FITC in DMF at 10mg/mL. Mix well to completely dissolve the FITC.
2. Add 15- to 20-fold molar excess of FITC to 0.5mL of antibody solution and immediately mix the reaction.
3. Incubate for 1 hour at room temperature in the dark.
4. Remove excess and hydrolyzed FITC by gel filtration, dialysis or with a Dye Removal Column.

**Procedure for Antibody Labeling with TRITC**

This method is adapted from Larsson.¹

**Materials Required**
- Conjugation Buffer: 100mM carbonate/bicarbonate buffer, pH 9.0
- Antibody solution: dialyze antibody into Conjugation Buffer at 6 mg/ml
- Device to remove excess dye, such as a Dye Removal Column (Product No. 22858), Zeba Spin Desalting Column (Product No. 89891) or Slide-A-Lyzer Dialysis Cassette, 10K MWCO (Product No. 66380)

**Procedure**
1. Dissolve TRITC in DMSO at 1mg/mL.
2. While stirring, slowly add 35µl of TRITC to 1mL of the 6mg/mL antibody solution and mix thoroughly.
3. Incubate for 2 hours at room temperature in the dark.
4. Remove excess and hydrolyzed TRITC by gel filtration, dialysis or with a Dye Removal Column.

**Additional Information**
- Visit our website for a listing of related products (other fluorophores and convenient labeling kits) and technical resources, such as the following Tech Tips:
  - Tech Tip #43: Protein stability and storage
  - Tech Tip #6: Extinction coefficients guide
  - Tech Tip #31: Calculate dye:protein (F/P) molar ratios
- Fading (photobleaching in tissue sections) sometimes can be reduced by mounting in an alkaline-buffered media (pH 9).¹ There are several reagents that may be used with FITC and/or TRITC derivatives to prevent fading, including n-propyl gallate at 0.1-0.25M dissolved in glycerol.² For FITC derivatives, o- or p-phenylenediamine added to the mounting buffer from 1µg/mL to 1mg/mL in glycerin also may be used.¹²
Related Thermo Scientific Products

- 28384 BupH™ Borate Buffer Packs, 40 packs
- 28372 BupH Phosphate Buffered Saline Packs, 40 packs
- 22858 Fluorescent Dye Removal Columns
- 66380 Slide-A-Lyzer Dialysis Cassettes, 10K MWCO
- 89889 Zeba Spin Desalting Columns, 7K MWCO, 2mL, 5 each
- 89890 Zeba Spin Desalting Columns, 7K MWCO, 2mL, 25 each
- 53029 Pierce™ NHS-Fluorescein Antibody Labeling Kit
- 53031 Pierce NHS-Rhodamine Antibody Labeling Kit
- 53027 Pierce FITC Antibody Labeling Kit
- 46409 NHS-Fluorescein, 1g
- 46410 NHS-Fluorescein, 100mg
- 46406 NHS-Rhodamine, 25mg
- 53024 DyLight™ 488 Antibody Labeling Kit
- 53025 DyLight 488 Microscale Antibody Labeling Kit

Cited References


General Reference


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