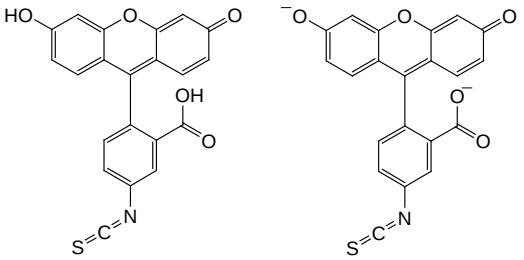
INSTRUCTIONS

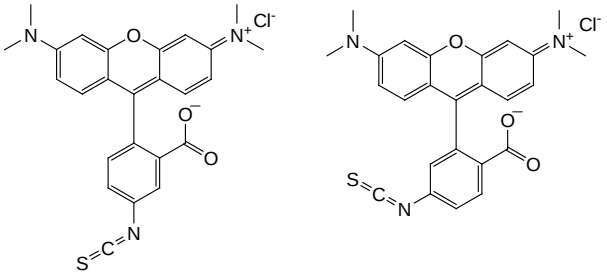
FITC and TRITC

46424 46425 46112

2081.1

Number	Description
46424	**FITC,** 1g
46425	**FITC,** 100mg Chemical name: Fluorescein isothiocyanate Molecular weight: 389.38 Extinction coefficient: 70,000M^{-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: 100,000M^{-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, sulfhydryl, 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.

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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.
2. Incubate for 2 hours at room temperature in the dark.
3. 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.[1,2]

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

1. Larsson, L. (1988). *Immunocytochemistry: Theory and Practice*. CRC. Boca Raton, **77-83**:224-5.
2. Goding, J. (1986). *Monoclonal Antibodies: Principles and Practice*, 2nd ed. Academic, London.

General Reference

1. Horisberger, M. (1984). In *Immunolabeling for Electron Microscopy*. Polak, J., Varndel, I. Ed. Elsevier: Amsterdam