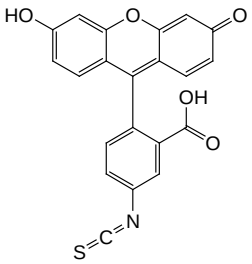
INSTRUCTIONS

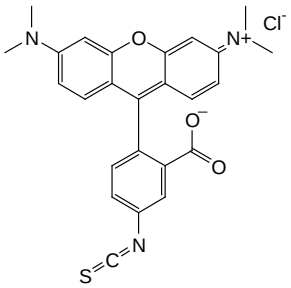
FITC and TRITC

46110 46112

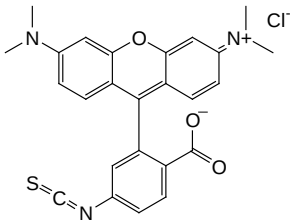
0365.2

Number	Description
46110	**Fluorescein Isothiocyanate (FITC),** 1 g Molecular Weight: 389.38 Exact Mass: 389.04 Extinction Coeff: 72,000 M^{-1} cm^{-1} (at 594 nm in aqueous buffer, pH 8) Ex/Em Wavelength : 494/520 nm CAS #3326-32-7
46112	**Tetramethylrhodamine-5-(and 6)-isothiocyanate (TRITC),** 10 mg Molecular Weight: 478.97 Exact Mass: 478.10 Extinction Coeff: 100,000 M^{-1} cm^{-1} (at 544 nm in methanol) Ex/Em Wavelength : 541/572 nm CAS #6749-36-6





and



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

Introduction

FITC and TRITC are among the most simple and commonly used reagents for protein fluorescent labeling. These isothiocyanates will crosslink to amino, sulfhydryl, imidazoyl, tyrosyl or carbonyl groups on a protein. 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. Avoid Tris buffer, which is a primary amine that will compete with the intended labeling reaction. Antibody and other proteins can be effectively labeled with several fluorophore tags per protein molecule when reacted with a 20- to 25-fold molar excess of isothiocyanate-activated fluorophore. Excess nonreacted and hydrolyzed reagent can be removed by dialysis or gel filtration.

Procedure for Labeling Streptavidin with FITC

This method was adapted from Horisberger.[1]

Materials Required

- Conjugation buffer: 100 mM carbonate/bicarbonate buffer, pH 9.0
- Streptavidin solution: dissolve 1 mg salt-free streptavidin (Product No. 21122) in 1 ml conjugation buffer
- Zeba™ Desalt Spin Column (Product No. 89891) or other gel filtration column with a 5,000-7,000 MW cut-off

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Procedure

1. Dissolve 200 µg of FITC in 200 µl of conjugation buffer and immediately mix it with 1 ml of streptavidin solution.

   Note: When substituting TRITC for FITC, a similar protocol can be followed; however, the TRITC must first be dissolved in dimethylsulfoxide (DMSO) at 100 µg/100 µl instead of 200 µg/200 µl in conjugation buffer.

2. Incubate for 1 hour at 37°C in the dark.
3. Remove excess and hydrolyzed FITC by gel filtration.

Procedure for Labeling an Antibody with FITC

Materials Required

- Conjugation buffer: 100 mM carbonate/bicarbonate buffer, pH 9.0
- Antibody solution: dissolve ~1 mg of antibody in 1 ml of conjugation buffer
- Zeba™ Desalt Spin Column (Product No. 89891) or other gel filtration column with a 5,000-7,000 MW cut-off

Procedure

1. Dissolve FITC in conjugation buffer at a final concentration of 1 mg/ml immediately before use.
2. Add 10 µl of FITC solution to the 1 ml of the antibody solution; mix thoroughly.
3. Incubate for 1 hour at room temperature in the dark.
4. Remove excess and hydrolyzed FITC by gel filtration.

Procedure for Labeling an Antibody with TRITC

This method is adapted from Larsson.[2]

Materials Required

- TRITC: dissolve in DMSO at 1 mg/ml
- Conjugation buffer: 100 mM carbonate/bicarbonate buffer, pH 9.0
- Antibody solution: dialyze antibody into conjugation buffer at 6 mg/ml
- Zeba™ Desalt Spin Column (Product No. 89891) or other gel filtration column with a 5,000-7,000 MW cut-off

Procedure

1. While stirring, slowly add 35 µl of TRITC to 1 ml of the 6 mg/ml antibody solution; mix thoroughly.
2. Incubate for 2 hours at room temperature in the dark.
3. Remove excess and hydrolyzed TRITC by gel filtration.

Additional Information

- Visit the web site for a listing of related Thermo Scientific products (other fluorophores and convenient labeling kits) and technical resources such as the Tech Tip: Calculate dye:protein (F/P) ratios.
- Fading (photobleaching in tissue sections) can sometimes be reduced by mounting in an alkaline buffered media (pH 9).[2] There are several reagents that may be used with FITC and/or TRITC derivatives to prevent fading including n-propyl gallate at 0.1-0.25 M dissolved in glycerol for FITC or TRITC.[3] For FITC derivatives, *o*- or *p*-phenylenediamine added to the mounting buffer from 1 µg/ml to 1 mg/ml in glycerin also may be used.[2,3]

References

1. Horisberger, M. (1984). In *Immunolabeling for Electron Microscopy*. Polak, J., Varndel, I. Ed. Elsevier: Amsterdam, **p. 98**.
2. Larsson, L. (1988). *Immunocytochemistry: Theory and Practice*. CRC. Boca Raton, **77-83**, 224-225.
3. Goding, J. (1986). *Monoclonal Antibodies: Principles and Practice*, 2nd ed. Academic, London.

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