Texas Red® Sulfonyl Chloride

46115

Number Description
46115 Texas Red Sulfonyl Chloride, 10 × 1 mg

Molecular Weight: 625.15
Excitation Wavelength: 596 nm
Emission Wavelength: 615 nm
Molar extinction coefficient at 596 nm in acetonitrile: 85,000 M⁻¹cm⁻¹
CAS # 82354-19-6

Storage: Upon receipt store desiccated at -20°C.

Introduction
Texas Red Sulfonyl Chloride is a long-wavelength derivative of rhodamine that is activated with sulfonyl chloride for covalent attachment to primary amines of antibodies, protein and other molecules. Texas Red Dye is compatible with multicolor microscopy and flow cytometry using instruments equipped with spectral line argon-krypton lasers. This reagent is also used in double labeling studies with fluorescein- or AMCA-conjugated samples.

Texas Red Dye is more pH-stable and photostable than fluorescein and rhodamine dyes. Texas Red Dye conjugates generally exhibit higher fluorescence quantum yield than those formed with other rhodamine dyes. In addition, Texas Red Dye is the least hydrophobic of the rhodamine derivatives, resulting in the lower background fluorescence in typical applications. An added advantage of Texas Red Sulfonyl Chloride over tetramethylrhodamine isothiocyanate (TRITC) for protein labeling, is that, during the conjugation procedure with Texas Red Sulfonyl Chloride, the excess sulfonyl chloride reagent hydrolyzes to form the extremely water-soluble sulforhodamine 101. This water-soluble by-product is easily removed from soluble the labeled protein by desalting or dialysis.

Sulfonyl chlorides react with primary amines (e.g., side chain of lysine residues in proteins) to form stable sulfonamides. Under varying conditions they also react with other nucleophiles including sulfhydryl groups (reduced cysteine), imidazoles (histidine), aliphatic hydroxyls (polysaccharides) and phenols (tyrosine). Conjugates of sulfonyl chlorides with thiol and imidazole groups are unstable, and conjugates with alcohols are subject to nucleophilic displacement.

Sulfonyl chlorides react with primary amines at pH 9-10 in phosphate, bicarbonate and borate buffers. Avoid reducing agents, primary amines and other strong nucleophiles in the coupling buffer.

Protocol for Labeling IgG with Texas Red Sulfonyl Chloride
The method described below is the same as that described by Titus, et al. (1982).

A. Materials
- Conjugation buffer: 0.1 M sodium carbonate/bicarbonate buffer, pH 9.0.
- Phosphate-buffered Saline (PBS): 20 mM sodium phosphate, 150 mM NaCl, pH 7.5.
- Texas Red Sulfonyl Chloride stock solution: Dissolve 1 mg Texas Red Sulfonyl Chloride in 50 µl anhydrous acetonitrile (prepare just before adding to the protein solution). Alternatively, add the Texas Red Sulfonyl Chloride directly to the protein solution as a solid powder. Do not dissolve this reagent in DMSO or store the reagent in solution (DMSO reacts with sulfonyl chlorides)(Boyle, 1986).
- Desalting column: Column containing 5 ml of gel-filtration resin having a 5,000 molecular weight cutoff.
B. Method

Note: Perform steps 1-3 on ice.

1. Dissolve 1-5 mg of protein in 1 ml of the chilled conjugation buffer.
2. Add 50 µl of the Texas Red Sulfonyl Chloride stock solution to the protein sample with rapid mixing.
3. Incubate the reaction mixture for 1 hour.
4. Dialyze the sample exhaustively or desalt the reaction mixture using a desalting column equilibrated with PBS or another suitable buffer. The purple Texas Red conjugated protein will appear in the void volume (the void volume of most desalting columns is approximately half the bed volume). The pink hydrolyzed reagent will appear following a colorless non-fluorescent fraction.

Protocol for Double Immunohistochemical Staining of Tissue Sections

Materials

- Cryostat tissue sections (human) fixed in acetone for 10 minutes and air-dried.
- Phosphate-buffered Saline (PBS): 20 mM sodium phosphate, 150 mM NaCl, pH 7.5.
- DSPBS (1): PBS containing 1.5% normal goat serum.
- DSPBS (2): PBS containing 1.5% normal horse serum.
- Primary antibody (1): Rabbit anti-human antibody to antigen #1 diluted with DSPBS (1) to appropriate concentration.
- Primary antibody (2): Mouse anti-human antibody to antigen #2 diluted with DSPBS (2) to appropriate concentration.
- Labeled secondary antibody (1): FITC-labeled goat anti-rabbit IgG diluted with DSPBS (1) to appropriate concentration.
- Labeled secondary antibody (2): Texas Red-labeled horse anti-mouse IgG diluted with DSPBS (2) to appropriate concentration.

Method

1. Rinse the human tissue section with PBS and incubate for 30 minutes at room temperature with DSPBS (1).
   Note: If paraffin sections are used in place of cryostat sections, first deparaffinate with xylene and rehydrate with descending ethanol washes. If picric acid was used during fixation, incubate overnight in PBS followed by several additional PBS washes to remove excess picric acid.
2. Incubate the tissue section with primary antibody (1) for 30 minutes in a humid environment at 37°C.
3. Rinse the tissue section 3 × 5 minutes with PBS.
4. Incubate the tissue section with labeled secondary antibody (1) for 30 minutes in a humid environment at 37°C
5. Rinse the tissue section 3 × 5 minutes with PBS.
6. Incubate the tissue section with DSPBS (2) for 30 minutes at room temperature.
7. Incubate the tissue section with primary antibody (2) for 30 minutes in a humid environment at 37°C.
8. Rinse the tissue section 3 × 5 minutes with PBS.
9. Incubate the tissue section with labeled secondary antibody (2) for 30 minutes in a humid environment at 37°C.
10. Rinse the tissue section 3 × 5 minutes with PBS.
11. Mount from distilled water onto submerged slides. Coverslip the section after it has dried. If the section is to be photographed, turn the slide upside down and place on absorbent paper. Use gentle pressure to expel excess mounting medium, wipe the edge of the slide, and seal the coverslip with clear nail polish. This will give better uniformity to the section and result in better photographs.

Technical Resources Available from Web Site

- Tech Tip #31: Calculate dye:protein (F/P) molar ratios
**Related Products**

Thermo Scientific DyLight™ Fluors are a newer class of high-intensity, photostable fluorescent dyes.

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**References**


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