

Texas Red[®] Sulfonyl Chloride

46115

0363.3

Number	Description
46115	Texas Red Sulfonyl Chloride , 1 × 10mg Molecular Weight: 625.15 Excitation Wavelength: 596nm Emission Wavelength: 615nm Molar extinction coefficient at 596nm in acetonitrile: 85,000 M ⁻¹ cm ⁻¹ CAS # 82354-19-6

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

Introduction

Thermo Scientific 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, proteins and other molecules. Texas Red Dye is compatible with multi-color 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 the soluble 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: 0.1M sodium carbonate/bicarbonate buffer, pH 9.0.
- Phosphate-buffered Saline (PBS): 20mM sodium phosphate, 150mM NaCl, pH 7.5.
- Texas Red Sulfonyl Chloride stock solution: Dissolve 1mg Texas Red Sulfonyl Chloride in 50μL 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 5mL of gel-filtration resin having a 5000kDa molecular-weight cutoff

B. Method

Note: Perform steps 1-3 on ice.

1. Dissolve 1-5mg of protein in 1mL of the chilled conjugation buffer.
2. Add 50 μ L 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): 20mM sodium phosphate, 150mM 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 \times 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 \times 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 \times 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 \times 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.

Additional Information on Our Website

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

Related Thermo Scientific Products

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

DyLight® Fluor Reagent and Kit Product Numbers

	Ex/Em (nm)	Amine-reactive NHS Ester		Sulfhydryl-reactive Maleimide
		Reagent (1mg/mL) and Kit	Reagent (5 x 50µg) and Kit	Reagent (1mg/mL)
DyLight 405	400/420	46400 and 53020	46401 and 53021	46600
DyLight 488	493/518	46402 and 53024	46403 and 53025	46602
DyLight 550	557/572	62262 and 84530	62263 and 84531	62290
DyLight 594	593/618	46412 and 53044	46413 and 53045	46608
DyLight 633	638/658	46414 and 53046	46417 and 53047	46613
DyLight 650	654/672	62265 and 84535	62266 and 84536	62295
DyLight 680	682/715	46418 and 53056	46419 and 53057	46618
DyLight 755	754/776	62278 and 84538	62279 and 84539	62298
DyLight 800	770/794	46421 and 53062	46422 and 53063	46621

General References

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