Revised: June 7, 2021

Product Information

Sulforhodamine 101 (Texas Red®)-PEO-3-Amine

Catalog Number: 90110

Unit Size: 5 mg

Storage and Handling
Store desiccated at 4°C or below and protect from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Stock solutions may be prepared in DMSO or DMF. Solutions can be stored protected from light at 4°C, for up to 12 months.

Molecular Information: $C_{41}H_{52}N_4O_9S_2$
Molecular Weight: 809
Color and Form: Dark purple solid
Solubility: Soluble in DMSO or DMF
Absorption/Emission: 583/603 nm

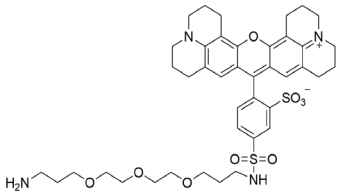

Figure 1. Sulforhodamine 101 (Texas Red®)-PEO-3-Amine

Product Description
Sulforhodamine 101(Texas Red®)-PEO-3-amine has a long water-soluble spacer separating the dye and the amine group. The dye can be coupled to activated carboxy groups or sulfonyl chlorides. EDC (EDAC) (Cat. no. 59002) may be used for direct coupling of carboxylic acids to primary amines using carbodiimide chemistry. Alternatively, EDC may be coupled with N-Hydroxysuccinimide (NHS) to prepare semi-stable NHS esters from carboxylate groups that can then be conjugated to primary amines. Visit our website to learn about our bright, photostable, and water-soluble CF® Dye amine derivatives, an excellent choice for fluorescent labeling.

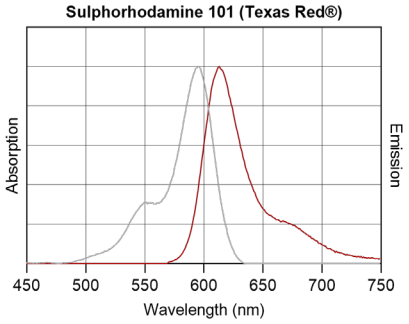

Figure 2. Absorbance and emission spectra of sulforhodamine 101 (Texas Red®).

References
1) Hermanson, G. (1996). Bioconjugate Techniques (1st ed.); 2) Biochim. Biophys. 200(3), 546(1970); 3) Biochim. Biophys. 160(2). 272(1968); 4) Anal. Biochem. 185(1), 131(1990); 5) Anal. Biochem. 156(1), 220(1986).

Direct EDC Coupling Protocol for Labeling Proteins with Dye Amine

EDC is a popular carbodiimide for conjugating carboxylate groups to primary amines between biological substances (1). EDC reacts with carboxylic acids to form a highly reactive, *O*-acylisourea intermediate. This intermediate can then react with a nucleophilic primary amine to form an amide bond. Please note that some side reactions may occur when using EDC with proteins. For instance, EDC can form a stable complex with exposed sulfhydryl groups and tyrosine residues (2,3). In addition, EDC may promote unwanted polymerization due to the presence of both amines and carboxylates on protein molecules. The following protocol has been adapted from literature for conjugating dye amines to proteins using EDC coupling (1,4,5). The protocol may be modified by changing the pH, buffer salts, and ratios of reactants to obtain the desired product.

Materials required but not provided
- Anhydrous DMSO (see Related Products)
- Reaction buffer: 0.1 M MES (2-[*N*-morpholino]ethanesulfonic acid), pH 4.7-6
- Protein sample in reaction buffer
- EDC (EDAC) (Cat. no. 59002)
- PBS buffer (pH 7.4)
- (Optional) Ultrafiltration Vial (see Related Products)

One-Step Labeling Protocol
1. Equilibrate EDC to room temperature.
2. Dissolve the protein to be modified in 200 uL of reaction buffer (see required materials above) for a final concentration of 20-100 uM.

   Note: Water or 0.1 M sodium phosphate, pH 7.3 may also be used. NaCl may also be added to the buffer if desired.
3. Add dye amine stock solution to protein solution in 10-fold molar excess. For example, 200 uL of 50 uM protein in reaction buffer is 10 nmole of protein total. Therefore add 100 nmole of dye amine (or 20 uL of 5 mM stock solution).
4. Add EDC to the reaction to obtain at least a 10-fold molar excess of EDC to the protein. Mix reaction well.

   Note: 0.5-0.1 M EDC in the reaction is usually a suitable concentration. For convenience, the reaction solution may be added to a tube containing 10 mg of EDC. If precipitation occurs, reduce the amount of EDC until the conjugate is soluble.
5. Incubate reaction for at least 2 hours at room temperature in the dark.
6. Separate the labeled protein from the free dye.

   a. Prepare a Sephadex® column (10 mm x 300 mm) equilibrated in PBS buffer (pH~7.4).

   b. After incubation, load the reaction solution onto the column and elute the column with PBS buffer. The first band eluted from the column corresponds to the antibody conjugate.

   Note: Choose a Sephadex® desalting medium most appropriate for the dye amine. For small scale labeling reactions, you may use an ultrafiltration vial (see Related Products) to remove the free dye from the conjugate in order to avoid an overly dilute product. Choose an ultrafiltration vial with a molecular weight cut-off at least 3X smaller than the protein molecular weight.
7. Store conjugate in an appropriate buffer and temperature for the protein of interest, protected from light.

Two-Stage EDC/Sulfo-NHS Coupling Protocol for Labeling Proteins with Dye Amine

The following protocol is a modified two-step protocol which involves activation of carboxyl proteins with EDC/sulfo-NHS and subsequent labeling with the dye amine (1,4). Sulfo-NHS improves the EDC coupling efficiency by increasing the stability of the *O*-acylisourea intermediate, thereby extending the half-life of the activated carboxylate to hours. The protocol involves activation at slightly acidic pH, which provides greater stability for the active ester intermediate. 2-Mercaptoethanol is also used to quench any unreacted EDC. The protocol may be modified by changing the pH, buffer salts, and ratios of reactants to obtain the desired product.

Materials required but not provided

- Anhydrous DMSO (see Related Products)
- Reaction buffer: 0.05 M MES (2-[*N*-morpholino]ethanesulfonic acid), 0.5 M NaCl, pH 6
- Protein sample in reaction buffer
- EDC (EDAC) (Cat. no. 59002)
- Sulfo-NHS (*N*-Hydroxysuccinimide)
- 2-mercaptoethanol
- PBS buffer (pH 7.4)
- (Optional) Hydroxylamine
- (Optional) Ultrafiltration Vial (see Related Products)
- (Optional) Sephadex® desalting column

Two-Step Labeling Protocol

1. Activation with EDC/Sulfo-NHS

1.1 Equilibrate EDC to room temperature.

1.2 Dissolve the protein to be modified in 200 uL of reaction buffer (pH 6.0) (see required materials above) for a final concentration of 20-100 uM.

1.3 Add EDC and sulfo-NHS to the solution for a final concentration of 2 mM EDC and 5 mM sulfo-NHS. Mix reaction well.

Note: To achieve accurate final concentrations, EDC and sulfo-NHS may be quickly dissolved in reaction buffer at higher concentrations, and then immediately pipetted into the protein solution to achieve the appropriate final concentrations.

1.4 Allow reaction to incubate for 15 minutes at room temperature.

1.5 Add 2-mercaptoethanol to the a reaction solution for a final concentration of 20 mM. Mix well and incubate at room temperature for 10 minutes.

Note: If the protein is sensitive to 2-mercaptoethanol, the activation may also be terminated by desalting (step 1.6).

1.6 Optional: Use a Sephadex® desalting column or equivalent to purify the activated protein.

Note: Choose a Sephadex® desalting medium most appropriate for the dye amine. The desalting process should be done rapidly to minimize hydrolysis and recover as much of the active ester as possible.

2. Labeling with dye amine

2.1 Add 10-fold molar excess of dye amine dissolved in concentrated PBS or other non-amine buffer to increase pH above 7.0. For example, 200 uL of 50 uM protein in reaction buffer is 10 nmole of protein total. Therefore add 100 nmole of dye amine (or 20 uL of 5 mM stock solution). Mix reaction well.

Note: The increase in pH above 7.0 is required to initiate the active ester reaction.

2.2 Incubate reaction for at least 2 hours at room temperature in the dark.

2.3 Optional: Quench the reaction by adding hydroxylamine to a final concentration of 10 mM. Mix reaction well.

Note: Alternative quenching reagents include 20-50 mM Tris, lysine, glycine and ethanolamine.

2.4 Separate the labeled protein from the dye amine.

a. Prepare a Sephadex® column (10 mm x 300 mm) equilibrated in PBS buffer (pH~7.4).

b. After incubation, load the reaction solution onto the column and elute the column with PBS buffer. The first band eluted from the column corresponds to the protein conjugate.

Note: For small scale labeling reactions, you may use an ultrafiltration vial (see Related Products) to remove the free dye from the conjugate in order to avoid an overly dilute product. Choose an ultrafiltration vial with a molecular weight cut-off at least 3X smaller than the protein molecular weight.

2.5 Store conjugate in an appropriate buffer and temperature for the protein of interest, protected from light.

Related Products

Catalog number	Product
92035... 96010	CF® Dye Amine
90106	Carboxyrhodamine 110-PEO3-amine, TFA salt, 5 isomer
90107	TAMRA-PEO3-amine, TFA salt, 5 isomer
90067	Biotin-PEO3-amine
90068	Biotin-PEO4-amine
90078	Biotin-PEO2-PPO2-amine, TFA salt
22004	Ultrafiltration Vial, 10K MWCO (5 per pack)
22018	Ultrafiltration Vial, 3K MWCO (5 per pack)
90082	DMSO, Anhydrous, 10 mL
59002	EDC (EDAC), 100 mg
22013	Bovine Serum Albumin, Fraction V, 50 g
22014	Bovine Serum Albumin, 30% Solution, 100 mL
41024-4L	Water, Ultrapure Molecular Biology Grade, 4 L

Please visit www.biotium.com to view our full selection of CF® reactive dyes and labeling kits, CF® dye labeled antibodies and other conjugates, and more.