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Product Information

TAMRA Succinimidyl Ester (SE)

Catalog no.	Dye	Unit size	Color and Form
90022	5(6)-TAMRA SE (mixed isomers)	25 mg	Red solid
90034	5-TAMRA SE	5 mg	Red solid
90035	6-TAMRA SE	5 mg	Red solid
90097	6-TAMRA SE in DMSO	100 uL	Red liquid

Storage and Handling

Store at -20°C desiccated and protected from light. Product is stable for at least one year from date of receipt when stored as recommended as a solid.

Stock solutions may be prepared in anhydrous DMSO or anhydrous DMF. Solutions can be aliquoted and stored with desiccant and protected from light at -20°C, for up to three months.

Molecular Information:

$C_{29}H_{25}N_3O_7$
Molecular Weight: 527.5
Absorption/Emission: 540/565 nm (MeOH)
A_{280}/A_{max} or C_f (protein): 0.3
Extinction Coefficient: 90,000

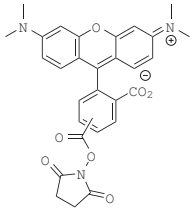

Figure 1. 5(6)-TAMRA SE (5-(and-6) Carboxytetramethylrhodamine succinimidyl ester).

Product Description

Carboxytetramethylrhodamine (TAMRA) is a commonly used red fluorescent dye, which can be optimally excited by a 546 nm laser line. The succinimidyl ester reacts readily with primary or secondary amines under mild conditions. Biotium offers very high grade 5-TAMRA SE and 6-TAMRA SE single isomers, and as a 5(6)-TAMRA SE mixed isomer.

Protocol for labeling IgG antibodies

The protocol below is a general labeling protocol for labeling IgG antibodies in sodium bicarbonate buffer. It is intended for experienced users and may require optimization to achieve the optimal degree of labeling (DOL) for your specific protein and dye. 1 umol dye is sufficient to label 8-15 mg IgG.

1. Materials required but not provided

- IgG: the IgG should be free of any amine-containing stabilizers, such as amino acids, Tris, BSA, or gelatin, as these substances will also react with the dye. Small molecules like Tris or amino acids can be removed by dialyzing the antibody against PBS buffer, or using an ultrafiltration vial to exchange the buffer (see related products). The presence of azide does not affect the labeling reaction.
- Anhydrous DMSO (90082)
- Sodium bicarbonate ($NaHCO_3$)
- Sephadex® G-25
- PBS buffer (pH ~7.4) (22020)
- Sodium azide (NaN_3)
- BSA (22013)

2. Labeling procedure

2.1 Prepare antibody solution for labeling

Dissolve the antibody in 0.1 M sodium bicarbonate buffer (pH ~8.3) at 2.5 mg/mL. If the IgG is already dissolved in a buffer such as PBS, the labeling solution can be prepared by adding one-tenth volume of 1 M sodium bicarbonate solution (pH 8.3) to the IgG solution for a final bicarbonate concentration of 0.1 M.

Note:
At about 2.5 mg/mL protein concentration, the labeling efficiency is generally around 35%. A protein concentration of less than 2.5 mg/mL is also suitable for labeling, although the labeling efficiency will be lower. A labeling efficiency of 20-30% can be expected with a protein concentration around 1 mg/mL. Even higher labeling efficiency is possible with protein concentration higher than 5 mg/mL. Because of variations in buffer and protein purity, accurate labeling efficiency can only be determined empirically under your exact conditions. If the IgG solution is too dilute, it may be concentrated using a 10K MWCO Ultrafiltration Vial (22004).

2.2 Prepare dye stock solution

Allow the vial of TAMRA SE to warm up to room temperature. Prepare a 10 mM dye stock solution in DMSO or DMF. Vortex the vial briefly to fully dissolve the dye, followed by brief centrifugation to collect the dye at the bottom of the vial.

Notes:
1) If the labeling reaction is to be carried out with a small amount of protein, the dye stock solution may need to be more dilute for accurate pipetting.

2) Unused stock solution may be stored at -20°C, protected from light and moisture. If anhydrous DMSO is used for making the solution, the dye should be stable for up to three months.

2.3 Carry out the labeling reaction

a) While stirring or vortexing the protein solution, add 15-25 uL of the 10 mM dye solution per mL of antibody solution in a dropwise fashion. These volumes correspond to dye/protein molar ratios between 9:1 to 15:1. Volume of dye added may need to be adjusted to achieve optimal DOL.

b) Continue to stir or rock the reaction solution at room temperature for 1 hour, protected from light.

Note:
While the labeling reaction is underway, proceed to Step 2.4a to prepare a Sephadex® column. TAMRA antibody conjugates are compatible with G-25 Sephadex® medium. A 10K MWCO Ultrafiltration Vial (22004) may also be used to remove free dye and concentrate IgG antibody. If you choose not to separate the labeled antibody from the free dye immediately after the reaction, you may add 50 uL of 1 M lysine to stop the reaction.

2.4 Separate the labeled protein from the free dye

a) Prepare a G-25 Sephadex® column (10 mm x 300 mm) equilibrated in PBS buffer (pH ~7.4).

b) After incubation, load the reaction solution from Step 2.3b onto the column and elute the column with PBS buffer. The first band excluded from the column corresponds to the antibody conjugate.

3. Determination of degree of labeling (DOL)

3.1 Determine the protein concentration

The concentration of the antibody conjugate can be calculated from the formula:
[conjugate] = {[A_{280} - (A_{max} x C_f)]/1.4} x dilution factor

where [conjugate] is the concentration of the antibody conjugate collected from the column in mg/mL; "dilution factor" is the fold of dilution used for spectral measurement; A_{280} and A_{max} are the absorbance readings of the conjugate at 280 nm and the absorption maximum respectively; C_f is the absorbance correction factor; and the value 1.4 is the extinction coefficient of IgG in mL/mg.

Note:
The protein solution eluted from the column may be too concentrated for accurate absorbance measurement and thus must be diluted to approximately ~0.1 mg/mL. The fold of dilution ("dilution factor") necessary can be estimated from the amount of starting antibody (i.e., 5 mg) and the total volume of the protein solution collected from the column.

3.2 Calculate the degree of labeling (DOL)

The DOL is calculated according to the formula:
DOL = (A_{max} x Mwt x dilution factor)/(ε x [conjugate])

where A_{max}, "dilution factor" and [conjugate] are as defined in Step 3.1, Mwt is the molecular weight of IgG (~150,000), and ε is the molar extinction coefficient of the dye. The optimal DOL (number of dyes conjugated to each protein) for single and mixed TAMRA SE isomers is 2-3. A DOL slightly above or below this range will also produce good results. If labeling a protein other than IgG, use the extinction coefficient for that specific protein.

4. Storage and handling of labeled antibody

For long-term storage, we recommend adding 5-10 mg/mL BSA and 0.01-0.03% sodium azide to the conjugate solution to prevent denaturation and microbial growth. The conjugate solution should be stored at 4°C and protected from light. If glycerol is added to a final concentration of 50%, the conjugate can be stored at -20°C. Under these conditions, antibody conjugates are stable for a year or longer.

Related Products

Catalog number	Product
80500	CF®Q520 Succinimidyl Ester
22004	Ultrafiltration Vial, 10K MWCO (5 per pack)
22018	Ultrafiltration Vial, 3K MWCO (5 per pack)
90082	DMSO, Anhydrous
22013	Bovine Serum Albumin, Fraction V
22014	Bovine Serum Albumin, 30% Solution
22020	10X Phosphate Buffered Saline (PBS)
41024-4L	Water, Ultrapure Molecular Biology Grade

Please visit our website at www.biotium.com to view our full selection of CF® dyes, our next-generation fluorescent dyes that have combined advantages in brightness, photostability, and water-solubility compared to other dyes such as Alexa Fluor®, DyLight®, Cy® Dyes or IRDyes®. We offer CF® dyes with a full selection of chemically reactive groups and easy-to-use labeling kits. We also offer a wide selection of CF® dye-labeleled antibodies and other bioconjugates.