Biotium

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Product Information

CF™ 770, Succinimidyl Ester(CF™ 770, SE)

Catalog Number: 92150

Unit Size: 1.0 µmole (sufficient for labeling 8-15 mg IgG)

Color and Form: Dark blue solid.

Storage and Handling

Store CF™770 SE desiccated at ≤ -20°C. When stored as directed, CF™770 SE should be stable for at least 6 months from the time of receipt.

Spectral Property

$\lambda_{abs}/\lambda_{em}$ = 770/797 nm (antibody conjugate in pH 7.4 buffer. See Figure 1 for spectra);

ε = ~220,000;

A_{280}/A_{max}, or CF = 0.06 (correction factor for estimating degree of protein labeling)

Solubility

Soluble in H_2O, DMF, DMSO or acetonitrile. For making stock solution, we recommend dissolving the dye in anhydrous DMSO (Biotium cat# 90082) at 10 mM.

Product Application

CF770 succinimidyl ester (CF770 SE) is an amine-reactive near-IR fluorescent dye. Following conjugation to protein, the dye has an absorption peak at 770 nm and emission peak at 797 nm. CF770 is significantly brighter than DyLight 800 or IRDye 800CW. Also importantly, CF770 is much more photostable than the other dyes. Because the long wavelength fluorescence of CF770 has excellent tissue penetration capability, the dye is ideal for in vivo imaging. Like our other near IR CF dyes, another unique feature of CF770 is that the dye is engineered to be minimally immunogenic. As a result, antibodies labeled with the dye are expected to have improved half-life during in vivo imaging.

Protocol for Labeling IgG antibodies

The protocol below is for labeling 5 mg of an IgG antibody. The procedure may be scaled up or down for any amount of protein as long as the ratios of the reagents are maintained.

1. Materials Required

- IgG: the IgG should be free of any amine-containing stabilizers, such as amino acids, or Tris, as these chemicals will also react with the dye. If these chemicals are present, the antibody should be dialyzed using PBS buffer (pH~7.4). Presence of azide does not affect the labeling reaction.
- CF™770 SE
- Sodium bicarbonate ($NaHCO_3$)
- Sephadex G-75 (fine or medium size)
- PBS buffer (pH~7.4)
- Sodium azide (NaN_3)
- BSA

2. Labeling Procedure

2.1 Prepare antibody solution for labeling.

Dissolve 5 mg of the antibody in about 2 mL 0.1 M sodium bicarbonate buffer (pH~8.3) to result in a labeling solution. If your IgG has been previously dissolved in a phosphate buffer, such as PBS buffer (must be free of any amine-containing chemicals- see Materials Required section), the labeling solution can be coveniently prepared by adding an appropriate amount of 1 M sodium bicarbonate solution (pH 8.3) to the IgG solution and adjusting the bicarbonate concentration to ~0.1 M. A protein concentration of less than 2.5 mg/mL is also suitable for the labeling although the labeling efficiency will be lower. A labeling efficiency of 20-30% can be expected with a protein concentration as low as about 1 mg/mL. At about 2.5 mg/mL protein concentration, the labeling efficiency is generally around 35%. Even higher labeling efficiency is possible with protein concentration higher than 5 mg/mL. Because of variations in buffer and protein purity, a more accurate labeling efficiency can only be determined under your exact condition. If the IgG solution is too dilute, it may be concentrated by ultrafiltration, such as by the use of a NanoSep™ ultrafiltration device (MWCO~10k) from Pall Corp.

2.2 Prepare dye stock solution

Allow a vial of CF™770 SE (1 umole) warm up to room temperature. Add 0.1 mL anhydrous DMSO (*e.g.*, Biotium Cat# 90082) to the vial to form a 10 mM dye stock solution. Vortex the vial briefly to fully dissolve the dye, followed by brief centrifugation to concentrate the dye at the bottom of the vial. If the labeling reaction is to be carried out with a much smaller amount of protein, the dye stock solution may need to be more dilute for accurate pipetting.

Note: 1) Any left-over stock solution may be stored at -20°C for later use. If anhydrous DMSO is used for making the solution, the dye should be stable for at least one month. 2) Dye stock solution may also be prepared in de-ionized water. However, because the dye will hydrolyze slowly, the stock solution in water should only be prepared immediately before the conjugation reaction and cannot be stored for later use.

2.3 Carry out the labeling reaction

a) While stirring or vortexing the protein solution, add 30-50 uL of the 10 mM dye stock in a dropwise fashion. The 30-50 uL dye volumes correspond to a dye/protein molar ratios of 9:1 to 15:1. As stated in Step 2.1, the dye/protein ratio may need to be higher for a more dilute protein solution because of the lower labeling efficiency for more dilute reactants. For IgG antibodies labeled with CF™770, the optimal DOL (number of dye conjugated to each protein) is from 3 to 5 although a DOL from 2 to 3 is also suitable.

Note: The optimal DOL here is for in vitro application. For in vivo imaging application, the optimal DOL may be different. However, because CF dyes are less immunogenic than other commercial near IR dyes, more dye per protein may be possible for higher fluorescence signal.

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

Important: while the labeling reaction is underway, proceed to the next step (Step 2.4a) to prepare a Sephadex G-75 column.

2.4 Separate the labeled protein from the free dye

a) Prepare a Sephadex G-75 column (10 mm x 300 mm) equilibrated in PBS buffer (pH~7.4).

b) Immediately 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.
Note: 1) Be sure to use Sephadex G-75, not Sephadex G-25, for the separation because Sephadex G-25 does not produce sufficient separation between the free dye and the labeled protein. For small scale labeling reaction, you may use a ultrafiltration device, such as a NanoSep™ ultrafiltration device (MWCO~10k) from Pall Corp, to remove the free dye from the conjugate in order to avoid overly dilute product.
2) 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.

3. Determination of Degree of Labeling

3.1 Determine the protein concentration

The concentration of the antibody conjugate can be calculated from the formula:

[conjugate] (mg/mL) = {[A_{280} - (A_{max} x CF)]/1.4} x dilution factor

where [conjugate] is the concentration of the antibody conjugate collected from the column; "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 (~770 nm for CF™770), respectively; CF is the absorbance correction factor (0.06 for CF™770); and the value 1.4 is the extinction coefficient of whole (H+L) 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 CF™770 (*i.e.*, ~220,000). For IgG antibodies with CF™770, the optimal DOL is 3-5 although a DOL from 2 to 3 will still produce good results.

4. Storage and Handling

For long-term storage, we recommend that BSA and sodium azide be added to the conjugate solution to final concentrations of 5-10 mg/mL and 0.01-0.03%, respectively, to prevent denaturation and microbial growth. The conjugate solution should be stored at 4 °C and protected from light.

*CF™ dye technology is covered by pending US and international patents.
** Alexa® is a registered trademark of Invitrogen, and Cy is a trademark of GE Healthcare; and DyLight is a trademark of Thermo Fisher Scientific..

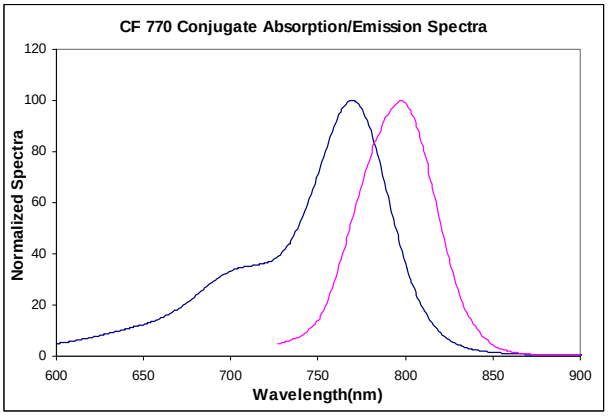

Figure 1. Absorption and emission spectra of CF™ 770 conjugated to goat anti-mouse IgG in PBS. Spectra of CF™ 770 conjugated to other proteins are similar.

Other Related Products

You may also be interested in the following related products:

Table 1. List of CF Dye Product Categories*

Product Type	Application	Unit Size	Unit Price($)
CF dye NHS esters	Labeling antibodies and other biomolecules	1umole	210.00
VivoBrite near-IR CF dye labeling kit for small animal in vivo imaging	Labeling antibodies for in vivo imaging	3 labelings	395.00
CF dye goat anti-mouse IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	130.00
CF dye goat anti-rabbit IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	130.00
CF dye goat anti-guinea pig IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	130.00
CF dye F(ab')2 fragment of goat anti-mouse IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.25 mL	105.00
CF dye F(ab')2 fragment of goat anti-rabbit IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.25 mL	105.00
Near-IR CF dye goat anti-mouse IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	150.00
Near-IR CF dye goat anti-rabbit IgG (H+L), 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.5 mL	150.00
Near-IR CF dye goat-anti-mouse IgG (H+L), highly cross-adsorbed, 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.25 mL	160.00
Near-IR CF dye goat-anti-rabbit IgG (H+L), highly cross-adsorbed, 2 mg/mL	Microscopy, flow cytometry and Western blotting	0.25 mL	160.00
CF dye annexin V conjugates, 50 ug/mL	Apoptosis	0.5 mL	210.00
CF dye phalloidin conjugates	F-actin staining	300 U	295.00

*For a complete list and descriptions of individual products, please visit biotium website: www.biotium.com