Biotium
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Product Information

CF™640R, NHS Ester (CF™ 640R, SE)

Catalog Number: 92108

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

Color and Form: Blue solid.

Storage and Handling

Store CF™640R SE desiccated at ≤ -20°C. When stored as directed, CF™640R SE should be stable for at least 6 months from the time of receipt.

Spectral Property

$\lambda_{abs}/\lambda_{em}$ = 642/662 nm (antibody conjugate in pH 7.4 buffer. See Figure 1 for spectra);

ε = ~105,000;

A_{280}/A_{max}, or CF = 0.37 (correction factor for estimating degree of protein labeling)

Solubility

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

Product Application

CF™ succinimidyl ester (CF™ SE) dyes are a series of amine-reactive fluorescent dyes designed for labeling proteins and other biomolecules. CF™ dyes are particularly useful for labeling antibodies. In many ways, CF™ dyes are superior to both Alexa® Fluor dyes and Cy™ dyes by having a combination of advantages in brightness, photostability, specificity and novel features ideal for in vivo imaging. CF™ dye-labeled antibodies give excellent signal-to-noise ratio without the need for using any blocking agent.

CF™640R is a rhodamine-based far-red fluorescent dye with excitation and emission maxima very similar to those of Cy™5 and Alexa Fluor® 647. CF™640R is much brighter than Cy™5 and at least as bright as Alexa Fluor® 647. A major advantage of CF™640R over Cy™ 5 and Alexa Fluor® 647 is its exceptional photostability. Cy™5 and Alexa Fluor® 647 are cyanine-based dyes and, like other cyanine dyes in general, have intrinsically poor photostability. CF™640R is also superior to Atto™ 647N, another spectrally similar dye frequently used in single-molecule imaging. The combination of excellent brightness and photostability makes CF™640R ideal for confocal microscopy, single-molecule imaging and other demanding applications based on fluorescence detection. Please see the CF™640R flyer for more information.

Protocol for Labeling IgG antibodies

The protocol below is for labeling 5 mg of an IgG antibody in bicarbonate buffer. Protocols for labeling other proteins can be modified accordingly. 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™640R SE
- Sodium bicarbonate ($NaHCO_3$)
- Sephadex G-50
- PBS buffer (pH~7.4)
- Sodium azide (NaN_3)
- BSA (IgG- and protease-free)

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 conveniently 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

Let a vial of CF™640R 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™640R, the optimal DOL (number of dye conjugated to each protein) is from 4-7 although a DOL slightly below or above this range will also produce good results.

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-50 column.

2.4 Separate the labeled protein from the free dye

a) Prepare a Sephadex G-50 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)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 (~642 nm for CF™640R), respectively; CF is the absorbance correction factor (0.37 for CF™640R); 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 CF™640R (i.e., ~105,000). For IgG antibodies with CF™640R, the optimal DOL is 4-7 although a DOL slightly above or below this range will also 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.

Absorption/Emission Spectra

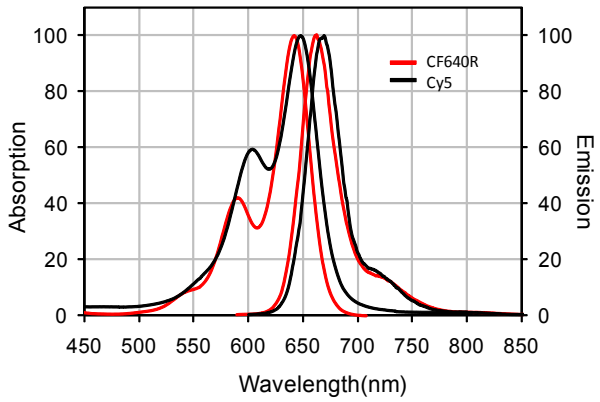

Figure 1. Normalized absorption and emission spectra for CF™640R and Cy™5 conjugated goat anti-mouse IgG.

Related Products

Cat.#	Product Name	Unit Size	
23001	EverBrite™ Mounting Medium	10 mL	
23002	EverBrite™ Mounting Medium with DAPI	10 mL	
23003	EverBrite™ Hardset Mounting Medium	10 mL	Coming Soon!
23004	EverBrite™ Harset Mounting Medium with DAPI	10 mL	Coming Soon!

*CF™ dye technology is covered by pending US and international patents.
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