XenoLight CF750 Succinimidyl Ester (SE)

Part Number: 125677

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

Color and Form: Dark blue solid.

Storage and Handling: Store XenoLight 750 SE desiccated at ≤ -20 °C. When stored as directed, XenoLight CF750 SE should be stable for at least 6 months from the time of receipt.

Spectral Property

$\lambda_{abs}/\lambda_{em}$ = 755/775 nm (antibody conjugate in pH 7.4 buffer; see figure 1 for spectra);

ε = ~250,000;

A_{280}/A_{max}, or CF = 0.03 (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.

Product Application

XenoLight CF750 succinimidyl ester is an amine-reactive near-IR fluorescent dye. When conjugated to protein, the dye has an absorption peak at 757 nm and emission peak at 777 nm. At a similar degree of labeling, XenoLight CF750-antibody conjugates can be expected to be more than twice as fluorescent and more photostable as antibody conjugates prepared with other similar dyes. Because of their long wavelength fluorescence, which has excellent tissue penetration and low interference from background fluorescence, XenoLight CF750 and our other near IR CF dyes are ideal for *in vivo* imaging. Another unique feature for all of our near IR CF dyes is that they are engineered to be minimally immunogenic. As a result, antibodies labeled with the dyes 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.
- XenoLight CF750 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 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® Corporation.

2.2 Prepare dye stock solution

Allow a vial of XenoLight CF750 SE (1 µmole) warm up to room temperature. Add 0.1 mL anhydrous DMSO 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:

i. 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.

ii. 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 µL of the 10 mM dye stock in a dropwise fashion. The 30-50 µL 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 CF750, 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.

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:

i. 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 Corporation, to remove the free dye from the conjugate in order to avoid overly dilute product.

ii. If you choose not to separate the labeled antibody from the free dye immediately after the reaction, you may add 50 µL 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 (~755 nm for XenoLight CF750), respectively; CF is the absorbance correction factor (0.09 for XenoLight CF750); 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 XenoLight CF750 (i.e., ~250,000). For IgG antibodies with XenoLight CF750, 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.

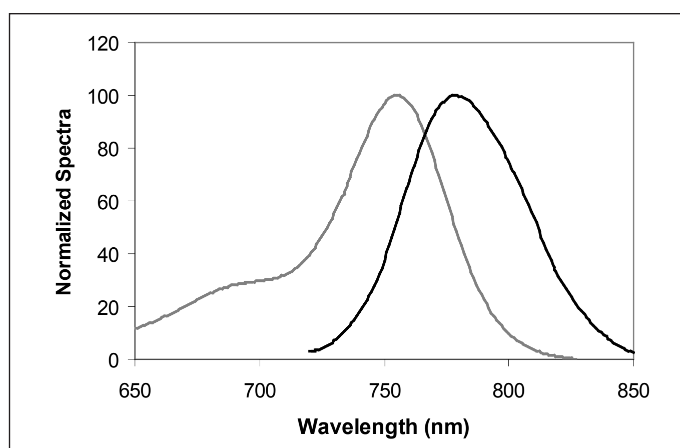

Figure 1. Normalized absorption and emission spectra of XenoLight CF750 conjugated goat anti-mouse IgG in pH 7.4 PBS buffer.

Caliper Life Sciences Offers the Following XenoLight Reagents

Catalog Number	Product Name	Unit size
122796	XenoLight D-Luciferin - K^+ Salt	1 g
125673	XenoLight CF680 Fluorescent Rapid Antibody Labeling Kit	3 labelings
125674	XenoLight CF750 Fluorescent Rapid Antibody Labeling Kit	3 labelings
125675	XenoLight CF770 Fluorescent Rapid Antibody Labeling Kit	3 labelings
125676	XenoLight CF680 succinimidyl ester, IR Fluorescent Dye	1 µmole
125677	XenoLight CF750 succinimidyl ester, IR Fluorescent Dye	1 µmole
125678	XenoLight CF770 succinimidyl ester, IR Fluorescent Dye	1 µmole
125964	XenoLight CF DiR	25 mg

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