Biotium
Glowing Products for Science™

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Product Information

CF™680 maleimide

Catalog Number: 92029

Unit Size: 1.0 μmole

Color and Form: Dark blue solid

Storage and Handling

Store CF™680 maleimide desiccated at ≤ -20°C. When stored as directed, CF™680 maleimide should be stable for at least 6 months from the time of receipt.

Technical Summary

Abs/Em Maxima: 681/698 nm (See Figure 1)

Extinction coefficient: 210,000

Molecular weight: 3363

A_{280}/A_{max} or CF: 0.09 (correction factor for estimating degree of protein labeling)

Direct replacement for: Alexa Fluor®680, Cy™5.5, DyLight 680

Solubility

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

Product Application

CF™680 maleimide reacts with thiol groups to form thioether-coupled products. The reaction can take place at pH 7 in the presence of amines. Under the neutral pH condition, the maleimide group does not react with histidine or arginine.

CF™680 maleimide is a near-IR fluorescent dye. When conjugated to protein, the dye has an absorption peak at 681 nm and emission peak at 698 nm. At a similar degree of labeling, CF680-antibody conjugates can be expected to be more than twice as fluorescent as antibody conjugates prepared from Alexa Fluor 680, IRDye 680, Dylight 680 or Cy5.5. Also importantly, CF680 is much more photostable than the other similar dyes. Because of their long wavelength fluorescence, which has excellent tissue penetration and minimizes interference from background fluorescence, CF680 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 proteins. Protocols for labeling other thiol-containing molecules are similar except for the purification procedures which may need to modified.

1. Materials Required but no Provided

- 10-100 mM phosphate (e.g., PBS), Tris or HEPES buffer with pH 7.0-7.5
- Sephadex G-75
- Anhydrous dimethylsulfoxide (DMSO, #90082) for preparing stock solution
- (optional) Tris-(2-carboxyethyl)phosphine (TCEP, #91049) for reducing disulfide binds in proteins to produce free thiol groups.
- BSA

2. Labeling Procedure

2.1 Prepare protein solution for labeling.

a) Dissolve the protein at 50-100 μM (7.5 mg/mL-15 mg/mL for IgG) in any of the mentioned buffers (See Materials section) at room temperature.

b) As an optional step, if you wish to free up more thiol groups from the disulfide bonds in the protein, you may add ~10-fold molar excess of TCEP at this stage. Incubate the reaction solution for ~30 min. The reduction reaction and the subsequent labeling reaction are best to be carried out in the presence of an inert gas (N_2 or Ar) to prevent re-formation of disulfide bonds.

2.2 Prepare dye stock solution

Let a vial of the CF™ maleimide (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 the dye stock to result in a dye/protein molar ratio of 10-20.

b) Continue to stir or rock the reaction solution at room temperature for 2 hour or at 4 °C overnight.

Tip: 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) 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: 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.

3. Determination of Degree of Labeling

3.1 Determine the protein concentration

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

$$[\text{conjugate}](\text{mg/mL}) = \left(\frac{(A_{280} - A_{max} \times C_f)}{1.4} \right) \times (\text{dilution factor})$$

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 (~681 nm for CF™680), respectively; C_f is the absorbance correction factor (0.09 for CF™680); 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 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:

$$\text{DOL} = \frac{A_{max} \times \text{Mwt} \times (\text{dilution factor})}{\varepsilon \times [\text{conjugate}]}$$

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™680 (i.e., ~210,000).

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.

Spectral Property

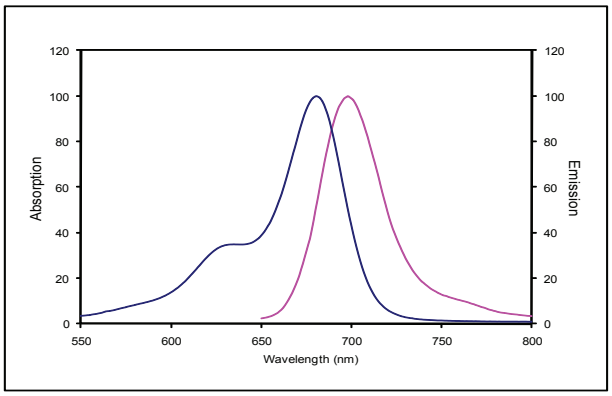

Figure 1. Normalized absorption and emission spectra of CF™680 conjugated goat anti-mouse IgG in pH 7.4 PBS buffer.

Other Related Products

A full selection of secondary antibodies, antibody labeling kits, and other bioconjugates including phalloidin, annexin V and α-bungarotoxin are available for many of our CF™ dyes. Please visit the Biotium website at www.biotium.com for details.

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