Biotium
Glowing Products for Science™

3159 Corporate place
Hayward, CA 94545
www.biotium.com

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Product Information

CF™640R maleimide

Catalog Number: 92034

Unit Size: 1.0 μmole

Color and Form: Dark Blue solid

Storage and Handling

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

Technical Summary

Abs/Em Maxima: 642/662 nm (See Figure 1)

Extinction coefficient: 105,000

Molecular weight: 954

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

Direct replacement for: Alexa Fluor®647, Cy5 and DyLight™647

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™640R 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™640R is a novel rhodamine-based far-red dye with excellent fluorescence quantum yield and exceptional photostability. The dye can be efficiently excited by the 633, 635, 640 or 647 nm laser with emission maximum at 650 nm. Both the excitation and emission wavelengths of CF™640R are only slightly shorter than those of the cyanine-based dye Cy™5 or Alexa Fluor®647 (Figure 1). Thus, CF™640R can be directly used to replace Alexa Fluor® 647 or Cy™5 in any application. CF™640R is much brighter than Cy™5 and at least as bright as Alexa Fluor® 647 when detected using optical settings optimized for either of the latter two dyes. The major benefit of using CF™640R, however, is the dye's superior photostability, which is simply unmatched by any cyanine-based far-red dyes, such as Alexa Fluor®647 and Cy™5.

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-25
- 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-25 column.

2.4 Separate the labeled protein from the free dye

a) Prepare a Sephadex G-25 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 (~642 nm for CF™640R), respectively; C_f 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 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™640R (i.e., ~105,000).

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

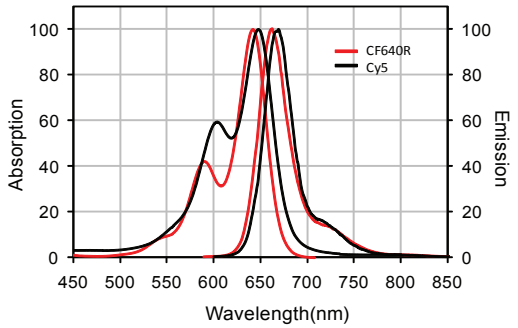

Figure 1. Absorption and emission spectra of CF™640R and Cy™5 conjugated to goat anti-mouse IgG in PBS.

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.
** Alexa® is a registered trademark of Invitrogen, and Cy is a trademark of GE Healthcare; and DyLight is a trademark of Thermo Fisher Scientific..