

# Product Information

## CF™660R, Succinimidyl Ester(CF™ 660R, SE)

**Catalog Number:** 92134

**Unit Size:** 1.0 μmole (sufficient for labeling 8-12 mg IgG)

**Color and Form:** Dark blue solid.

### Storage and Handling

Store CF™660R SE desiccated at ≤ -20°C. When stored as directed, CF™660R SE should be stable for at least 6 months from the time of receipt.

### Technical Summary

**Abs/Em Maxima:** 663/682 nm (See Figure 1)

**Extinction coefficient:** 100,000

**Molecular weight of free acid form:** 888

**A<sub>280</sub>/A<sub>max</sub> or CF:** 0.51 (correction factor for estimating degree of protein labeling)

**Flow cytometry laser line:** 633 or 635 nm

**Microscopy laser line:** 633 or 635 nm

**Direct replacement for:** Alexa Fluor® 660, Allophycocyanin (APC)

### Product Application

CF™660R succinimidyl ester (CF™660R SE) is for labeling proteins or other biomolecules having an amine group. The succinimidyl ester group of the dye reacts with an amine group to form a stable amide linkage. CF™660R dye is a novel rhodamine-based dye having good fluorescence quantum yield and exceptional photostability (Download CF™660R flyer at Biotium website for more information). The dye is also highly water-soluble and pH-insensitive. CF™660R is ideal for confocal microscopy or any applications that demand both brightness and photostability.

The protocol below is a typical procedure for labeling IgG antibodies in bicarbonate buffer. Protocols for labeling other proteins can be modified accordingly.

### 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™660R SE
- Sodium bicarbonate (NaHCO<sub>3</sub>)
- Sephadex G-25
- PBS buffer (pH~7.4)
- Sodium azide (NaN<sub>3</sub>)
- 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 Corp.

#### 2.2 Prepare dye stock solution

Let a vial of CF™660R SE (1 μmole) 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 μL of the 10

mM dye stock in a dropwise fashion. The 30-50  $\mu$ L 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<sup>TM</sup>660R, 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 acceptable results.

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

**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) Immediately load the reaction solution from Step 2.3b onto the column and elute the column with 1X 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<sup>TM</sup> 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  $\mu$ L of 1 M lysine to stop the reaction. In most cases, this step may not be necessary as any unconjugated dye should have already been fully hydrolyzed by the end of 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:

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

where [conjugate] is the concentration of the antibody conjugate collected from the column; "dilution factor" is the fold of dilution used for spectral measurement (See Note below);  $A_{280}$  and  $A_{\text{max}}$  are the absorbance readings of the conjugate at 280 nm and the absorption maximum (~663 nm for CF<sup>TM</sup>660R), respectively;  $C_f$  is the absorbance correction factor (0.51 for CF<sup>TM</sup>660R); 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:

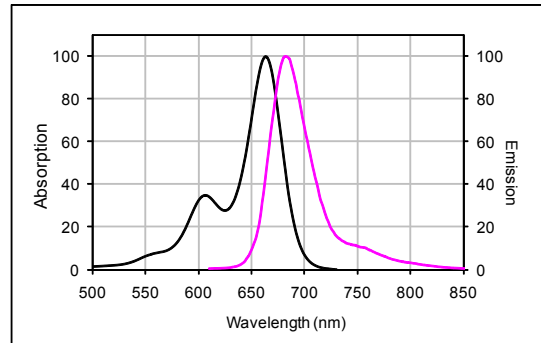
$$\text{DOL} = \frac{A_{\text{max}} \times \text{Mwt} \times (\text{dilution factor})}{\epsilon \times [\text{conjugate}]}$$

where  $A_{\text{max}}$  "dilution factor" and [conjugate] are as defined in Step 3.1, Mwt is the molecular weight of IgG (~150,000), and  $\epsilon$  is the molar extinction coefficient of CF<sup>TM</sup>660R (*i.e.*, 100,000). For IgG antibodies with CF<sup>TM</sup>660R, 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 of CF<sup>TM</sup>660R Conjugated Antibodies



**Figure 1.** Absorption and emission spectra of CF<sup>TM</sup>660R 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  $\alpha$ -bungarotoxin are available for many of our CF<sup>TM</sup> dyes. Please visit the Biotium website at [www.biotium.com](http://www.biotium.com) for details.

\*CF<sup>TM</sup> dye technology is covered by pending US and international patents.