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Product Information

CF™660R, Succinimidyl Ester

Catalog Number **SCJ4600053**
Storage Temperature –20 °C

TECHNICAL BULLETIN

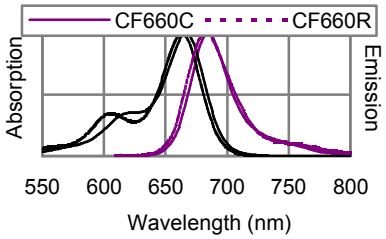
Product Description

CF™660R, succinimidyl ester (CF660R SE) is used 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.

CF660C and CF660R are two spectrally similar fluorescent dyes that emit fluorescence at ~685 nm in the borderline spectral region between far-red and near-IR. Although their absorption maxima are around 660 nm, both dyes can be sufficiently excited by the 633 or 635 nm laser. When combined with other CF dyes of shorter wavelengths, CF660C or CF660R can serve as a useful long wavelength dye in multicolor detection applications. The two dyes are spectrally similar to Alexa Fluor® 660 but are far superior to the latter in performance. Like Alexa Fluor 660, CF660C is a cyanine-based dye. However, when conjugated to protein, CF660C is several fold brighter and significantly more photostable than Alexa Fluor 660.

CF660R dye properties:
Abs/Em Maxima: 663/682 nm (See Figure 1)
Extinction coefficient: 100,000
Molecular mass of free acid: ~888
A_{280}/A_{max} or CF (correction factor for estimating degree of protein labeling): 0.51
Flow cytometry laser line: 633, 635, or 640 nm
Microscopy laser line: 633, 635, or 640 nm
Direct replacement for: Alexa Fluor 660

Figure 1.
Absorption and emission spectra of CF660R conjugated to goat anti-mouse IgG in PBS.



Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the dye desiccated at –20 °C. When stored as directed, the dye should remain active for at least 6 months.

Procedure

This procedure is a guideline for labeling IgG antibodies in bicarbonate buffer. Procedures for labeling other proteins can be modified accordingly. The procedure is for labeling 5 mg of an IgG antibody in bicarbonate buffer. The procedure may be scaled up or down for any amount of protein as long as the ratios of the reagents are maintained. One μmole of CF660R SE (dark blue solid) is provided, which is sufficient for labeling 8–12 mg of IgG.

Reagents Required but Not Provided

- IgG: The IgG should be free of any amine-containing compounds, such as amino acids or Tris buffer, 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.
- Sodium bicarbonate ($NaHCO_3$)
- Sephadex® G-25
- PBS buffer, pH ~7.4
- Sodium azide (NaN_3)
- BSA

Antibody Preparation

Dissolve 5 mg of the antibody in ~2 mL of 0.1 M sodium bicarbonate buffer, pH ~8.3, resulting in the Labeling Solution. If the IgG has been previously dissolved in phosphate buffer, such as PBS buffer (must be free of any amine-containing chemicals), the Labeling Solution can be conveniently prepared by adding an appropriate volume of 1 M sodium bicarbonate solution, pH 8.3, to the IgG solution and adjusting the bicarbonate concentration to ~0.1 M. If the IgG solution is too dilute, it may be concentrated by ultrafiltration.

The labeling efficiency of the dye reaction decreases with decreasing protein concentration. A labeling efficiency of 20–30% can be expected with a protein concentration as low as ~1 mg/mL. At ~2.5 mg/mL protein concentration, the labeling efficiency is generally ~35%. Even higher labeling efficiency is possible with protein concentration >5 mg/mL. Because of variations in buffer and protein purity, a more accurate labeling efficiency must be determined empirically.

Dye Stock Solution Preparation

Let a vial of CF660R SE (1 μmole) warm up to room temperature. Add 0.1 mL of anhydrous DMSO to the vial, forming a 10 mM Dye Stock Solution. Vortex the vial briefly to fully dissolve the dye, followed by brief centrifugation to collect the solution 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.

Notes: Any remaining 10 mM Dye Stock Solution may be stored at –20 °C for later use. If anhydrous DMSO is used for making the solution, the dye should remain active for at least one month.

The dye stock solution may also be prepared in 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

Labeling Reaction

1. While stirring or vortexing the Labeling Solution, add 30–50 μL of the 10 mM Dye Stock Solution in a dropwise fashion. The 30–50 μL volume corresponds to a dye:protein molar ratio of 9:1 to 15:1. As stated earlier, 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 CF660R, the optimal degree of labeling (DOL, number of dye molecules conjugated to each protein molecule) is from 4–7, although a DOL slightly below or above this range will also produce acceptable results.

2. Continue to stir or rock the Reaction Solution at room temperature for 1 hour.

Note: While the labeling reaction is underway, prepare a Sephadex G-25 column for reaction clean-up.

Reaction Clean-up - Separation of the labeled protein from the free dye

1. Prepare a Sephadex G-25 column (10 mm × 300 mm) equilibrated in PBS buffer, pH ~7.4.
2. Immediately load the Reaction Solution onto the column and elute the column with 1× PBS buffer. The first band excluded from the column corresponds to the antibody conjugate.

Notes: For a small scale labeling reaction, an ultrafiltration device may be used to remove the free dye from the conjugate in order to avoid an overly dilute conjugate solution.

Instead of separating the labeled antibody from the free dye immediately after the reaction, one may add 50 μL of 1 M lysine solution 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.

Storage and Handling

For long-term storage and to prevent denaturation and microbial growth, the addition of BSA and sodium azide to the conjugate solution is recommended to final concentrations of 5–10 mg/mL and 0.01–0.03%, respectively. The conjugate solution should be stored at 2–8 °C and protected from light.

Results

Determine the protein concentration

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

$$[\text{conjugate}] = \{[A_{280} - (A_{max} \times CF)]/1.4\} \times df \quad (\text{mg/mL})$$

[conjugate] (mg/mL) - concentration of the antibody conjugate collected from the column

df (dilution factor) - the fold of dilution used for spectral measurement (See Note)

A_{280} and A_{max} are the absorbance readings of the conjugate at 280 nm and the absorption maximum (~663 nm for CF660R), respectively

CF - the absorbance correction factor (0.51 for CF660R)

1.4 - the extinction coefficient of IgG in mL/mg.

Note: The protein solution eluted from the column may be too concentrated for an accurate absorbance measurement and thus, must be diluted to ~0.1 mg/mL. The fold of dilution (df, 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.

Calculate the degree of labeling (DOL)

The DOL is calculated according to the formula:

$$DOL = (A_{max} \times Mwt \times df)/(\varepsilon \times [\text{conjugate}])$$

A_{max}, df (dilution factor), and [conjugate] are as defined in determination of protein concentration

Mwt - molecular mass of IgG (~150,000)

ε - molar extinction coefficient of CF660R (*i.e.*, 100,000).

For IgG antibodies labeled with CF660R, the optimal DOL is 4-7, although a DOL slightly above or below this range will also produce acceptable results.

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