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Product Information

CF™680, Maleimide

Catalog Number **SCJ4600054**
Storage Temperature –20 °C

TECHNICAL BULLETIN

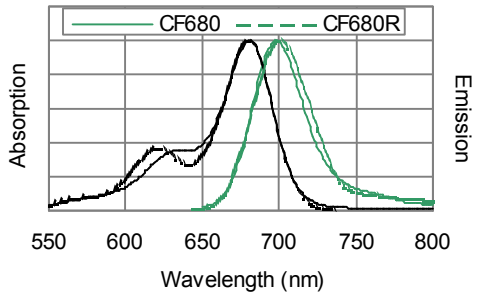
Product Description

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

CF680 and CF680R are two outstanding near-IR dyes excitable at ~680 nm with emission at ~700 nm. The two dyes each have unique properties suitable for different application needs. CF680 is a highly water-soluble cyanine-based dye with a molecular weight of ~3,000. This dye is excellent for labeling antibodies, producing the brightest fluorescence and highest signal-to-noise ratio in immunostaining among spectrally similar dyes, such as Cy™5.5, Alexa Fluor® 680, DyLight® 680 and IRDye® 680. However, because of its relatively large molecular size, CF680 is not recommended for labeling nucleic acids or relatively small biomolecules, for which our CF680R is better suited.

CF680 dye properties:
Abs/Em Maxima: 681/698 nm (See Figure 1)
Extinction coefficient: 210,000
Molecular weight: ~3,363
A_{280}/A_{max} or CF (correction factor for estimating degree of protein labeling): 0.09
Flow cytometry laser line: 633, 635, or 640 nm
Microscopy laser line: 633, 635, or 640 nm
Direct replacement for: Alexa Fluor 680, Cy5.5, DyLight 680, and IRDye 680LT

Figure 1.
Absorption and emission spectra of CF680 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

The protocol below is for labeling proteins. Protocols for labeling other thiol-containing molecules are similar except for the purification procedures, which may require modification.

Reagents Required but Not Provided

- 10–100 mM phosphate (e.g., PBS), Tris, or HEPES buffer with pH 7.0–7.5
- Sephadex® G-25
- Anhydrous dimethylsulfoxide (DMSO) for preparing stock solution
- (Optional) Tris-(2-carboxyethyl)phosphine (TCEP) for reducing disulfide binds in proteins to produce free thiol groups.
- BSA

Protein Preparation

Dissolve the protein at 50–100 mM in any of the buffers listed under Reagents Required at room temperature. As an optional step, you may add ~10-fold molar excess of TCEP at this stage to reduce disulfide bonds and increase the number of thiol groups available for labeling. Incubate the protein with TCEP 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.

Dye Stock Solution Preparation

Warm a vial of the CF680 maleimide (1 μmole) to room temperature. Add 0.1 mL 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 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 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.

Labeling Reaction

1. While stirring or vortexing the protein solution, add the dye stock to result in a dye/protein molar ratio of 10–20.

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

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.

Note: 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.

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:

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

[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 (~681 nm for CF680), respectively

CF - the absorbance correction factor (0.09 for CF680)

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:

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

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 CF680 (*i.e.*, 210,000).

For IgG antibodies labeled with CF680, the optimal DOL is from 3-5, although a DOL of 2-3 will also produce acceptable results.

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