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Product Information

CF™488A, Maleimide

Catalog Number **SCJ4600016**
Storage Temperature –20 °C

TECHNICAL BULLETIN

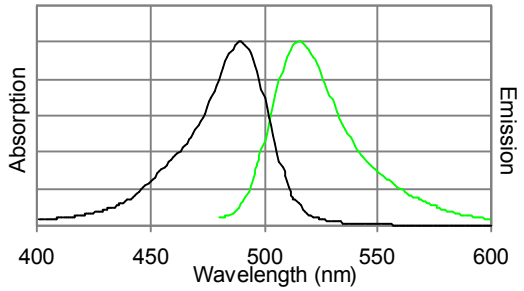
Product Description

CF™488A 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.

CF488A is a green fluorescent dye optimally excitable by the 488 nm argon laser line. Under common detection conditions, CF488A is at least as bright as Alexa Fluor® 488. However, a major advantage of CF488A over Alexa Fluor 488 is that antibody conjugates prepared from the former are biologically more specific. Alexa Fluor 488 carries multiple negative charges, which can significantly change the isoelectric point of the proteins the dye labels and consequently alter the specificity of the protein conjugates. CF488A, on the other hand, is minimally charged. Thus, antibody conjugates prepared from the dye ensure biological detection with high signal-to-noise ratio. Another feature of CF488A is the emission peak wavelength is about 10 nm shorter than that of Alexa Fluor 488 and 15 nm shorter than that of the traditional green dye FITC (or FAM). The shorter wavelength of CF488A offers the advantage of less fluorescence “spill-over” in the red channel in multi-color detection applications.

CF488A dye properties:
Abs/Em Maxima: 490/515 nm (See Figure 1)
Extinction coefficient: 70,000
Molecular weight: ~1036
A_{280}/A_{max} or CF (correction factor for estimating degree of protein labeling): 0.1
Flow cytometry laser line: 488 nm
Microscopy laser line: 488 nm
Direct replacement for: Alexa Fluor 488, Cy™2, DyLight® 488, FAM, and fluorescein (FITC)

Figure 1.
Absorption and emission spectra of CF488A 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 minutes. 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 CF488A 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:

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

[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 (~490 nm for CF488A), respectively

CF - the absorbance correction factor (0.1 for CF488A)

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 CF488A (*i.e.*, 70,000).

For IgG antibodies labeled with CF488A, the optimal DOL is 7-9, although a DOL slightly above or below this range will also produce acceptable results.

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