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Product Information

CF™488A, Succinimidyl Ester

Catalog Number **SCJ4600018**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

CF™488A, succinimidyl ester (CF488A 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.

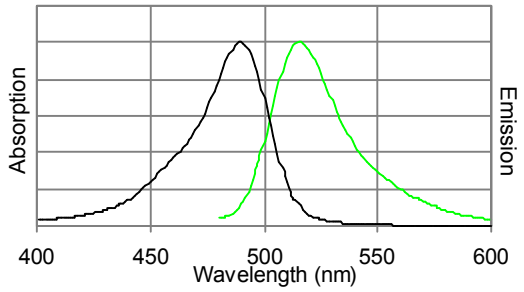
CF488A is a green fluorescent dye optimally excitable by the 488 nm argon laser line. Under common detection conditions, CF488A is at least as bright as Alexa Fluor® 488. However, a major advantage of CF488A over Alexa Fluor 488 is that antibody conjugates prepared from the former are biologically more 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 that 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 mass of free acid: ~914
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)

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.

Figure 1.
Absorption and emission spectra of CF488A conjugated to goat anti-mouse IgG in PBS.



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 CF488A SE (orange 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 CF488A 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 CF488A, the optimal degree of labeling (DOL, number of dye molecules conjugated to each protein molecule) is from 7–9, 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 (~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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