Product Information

CF® Dye Amine

See product page for a full list of product names, unit sizes, and catalog numbers.

Storage and Handling

Store at -20°C, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended. Stock solutions may be prepared in DMSO or water and can be stored at ≤ -20°C for at least 12 months.

Product Technical Information

See product page for spectral properties and other dye-specific technical information. See our Spectra Viewer to view and download the dye excitation and emission spectra.

Product Description

CF® Dye Amines can be conjugated to activated carboxylic acids in proteins or other molecules using carbodiimide chemistry. EDC (EDAC) (Cat. No. 59002) is a popular carbodiimide for direct coupling of carboxylate groups to primary amines between biological substances (1). EDC reacts with carboxylic acids to form a highly reactive, *O*-acylisourea intermediate. This intermediate can then react with a nucleophilic primary amine to form an amide bond.

Alternatively, EDC may be coupled with *N*-Hydroxysuccinimide (NHS) to prepare semi-stable NHS esters from carboxylate groups that can then be conjugated to CF® Dye Amines (1,2). Sulfo-NHS, the water-soluble analog of NHS, improves the EDC coupling by increasing the efficiency of formation of the *O*-acylisourea intermediate in water.

Our CF® Dye Amines are bright, photostable, and water-soluble, making them an excellent choice for fluorescent labeling. CF® Dye Amines are available in a range of dye colors, from UV to near-infrared.

Experimental Protocols

Direct EDC coupling protocol for labeling proteins with CF® Dye Amines

The following protocol has been adapted from literature for conjugating CF® Dye Amines to proteins using EDC coupling (1-3). The protocol may be modified by changing the pH, buffer salts, and ratios of reactants to obtain the desired product.

Please note that some side reactions may occur when using EDC with proteins. For instance, EDC can form a stable complex with exposed sulfhydryl groups and tyrosine residues (4,5). In addition, EDC may promote unwanted polymerization due to the presence of both amines and carboxylates on protein molecules.

Materials required but not provided

- Anhydrous DMSO (Cat. No. 90082)
- Reaction Buffer: 0.1 M MES (2-[*N*-morpholino]ethanesulfonic acid), pH 4.7-6
- EDC (EDAC) (Cat. No. 59002)
- PBS buffer, pH 7.4
- (Optional) Ultrafiltration vial (see Related Products)
- Sephadex®; see product page for the appropriate type of Sephadex® for each CF® Dye

One-step labeling protocol

1. Equilibrate EDC to room temperature.
2. Dissolve the protein to be modified in 200 uL of Reaction Buffer for a final concentration of 20-100 uM.

   Note: Water or 0.1 M sodium phosphate, pH 7.3 may be used instead. NaCl may also be added to the buffer if desired.
3. Add CF® Dye Amine stock solution to protein solution in 10-fold molar excess. For example, 200 uL of 50 uM protein in reaction buffer is 10 nmol of protein total. Therefore, add 100 nmol of CF® Dye Amine (or 20 uL of 5 mM stock solution).
4. Add EDC to the reaction to obtain at least a 10-fold molar excess of EDC to the protein. Mix reaction well.

   Note: 0.1-0.5 M EDC in the reaction is usually a suitable concentration. For convenience, the reaction solution may be added to a tube containing 10 mg of EDC. If precipitation occurs, reduce the amount of EDC until the conjugate is soluble.
5. Incubate reaction for at least 2 hours at room temperature in the dark.

6. Separate the labeled protein from the free dye.

   a. Prepare a Sephadex® column (10 mm x 300 mm) equilibrated in PBS buffer (pH ~7.4).

   b. After incubation, load the reaction solution onto the column and elute the column with PBS buffer. The first band eluted from the column corresponds to the antibody conjugate.

   Note: See product page for the appropriate Sephadex® medium to use for each CF® Dye. For small scale labeling reactions, you may use an ultrafiltration vial (see Related Products) to remove the free dye from the conjugate in order to avoid an overly dilute product. Choose an ultrafiltration vial with a molecular weight cut-off at least 3X smaller than the protein molecular weight.

7. Store conjugate in an appropriate buffer and temperature for the protein of interest, protected from light.

Two-step EDC/sulfo-NHS coupling protocol for labeling proteins with CF® Dye Amines

The following protocol is a modified two-step protocol that involves activation of carboxyl proteins with EDC/sulfo-NHS and subsequent conjugation with CF® Dye Amines (1,2). Activation is performed at an acidic pH, which provides greater stability for the active ester intermediate. 2-Mercaptoethanol is used to quench any unreacted EDC. The protocol may be modified by changing the pH, buffer salts, and ratios of reactants to obtain the desired product.

Materials required but not provided

- Anhydrous DMSO (Cat. No. 90082)
- Reaction Buffer: 0.05 M MES (2-[*N*-morpholino]ethanesulfonic acid), 0.5 M NaCl, pH 6
- EDC (EDAC) (Cat. No. 59002)
- Sulfo-NHS (*N*-Hydroxysuccinimide)
- 2-Mercaptoethanol
- PBS buffer, pH 7.4
- (Optional) Hydroxylamine
- (Optional) Ultrafiltration vial (see Related Products)
- Sephadex®; see product page for the appropriate type of Sephadex® for each CF® Dye

Two-step labeling protocol

1. Equilibrate EDC to room temperature.
2. Dissolve the protein to be modified in 200 uL of Reaction Buffer for a final concentration of 20-100 uM.
3. Add EDC and sulfo-NHS to the solution for a final concentration of 2 mM EDC and 5 mM sulfo-NHS. Mix reaction well.

   Note: To achieve accurate final concentrations, EDC and sulfo-NHS may be quickly dissolved in reaction buffer at higher concentrations, and then immediately pipetted into the protein solution to achieve the appropriate final concentrations.
4. Incubate reaction for 15 minutes at room temperature.
5. Add 2-mercaptoethanol to the reaction solution for a final concentration of 20 mM. Mix well and incubate at room temperature for 10 minutes.

   Note: If the protein is sensitive to 2-mercaptoethanol, the activation may also be terminated by desalting (step 6).
6. Optional: Use a Sephadex® G-25 desalting column or equivalent to purify the activated protein.

   Note: The desalting process should be done rapidly to minimize hydrolysis and recover as much of the active ester as possible.
7. Add 10-fold molar excess of CF® Dye Amine dissolved in concentrated PBS or other non-amine buffer to increase pH above 7.0. For example, 200 uL of 50 uM protein in reaction buffer is 10 nmol of protein total. Therefore, add 100 nmol of CF® Dye Amine (or 20 uL of 5 mM stock solution). Mix reaction well.

   Note: The increase in pH above 7.0 is required to initiate the active ester reaction.
8. Incubate reaction for at least 2 hours at room temperature in the dark.
9. Optional: Quench the reaction by adding hydroxylamine to a final concentration of 10 mM. Mix reaction well.

   Note: Alternative quenching reagents include 20-50 mM Tris, lysine, glycine, and ethanolamine.
10. Separate the labeled protein from the dye.

    a. Prepare a Sephadex® column (10 mm x 300 mm) equilibrated in PBS buffer (pH ~7.4).

    b. After incubation, load the reaction solution onto the column and elute the column with PBS buffer. The first band eluted from the column corresponds to the protein conjugate.

    Note: See product page for the appropriate Sephadex® medium to use for each CF® Dye. For small scale labeling reactions, you may use an ultrafiltration vial (see Related Products) to remove the free dye from the conjugate in order to avoid an overly dilute product. Choose an ultrafiltration vial with a molecular weight cut-off at least 3X smaller than the protein molecular weight.
11. Store conjugate in an appropriate buffer and temperature for the protein of interest, protected from light.

Related Products

Cat. No.	Product
22004	Ultrafiltration Vials, 10K MWCO
22018	Ultrafiltration Vials, 3K MWCO
90082	DMSO, Anhydrous
59002	EDC (EDAC)
92103... 97502	CF® Dye SE/TFP Ester
92020... 96079	CF® Dye Maleimide
92096... 92099	CF® Dye MTS
92050... 92059	CF® Dye Aminooxy
92151... 96064	CF® Dye Hydrazide
92170... 96128	CF® Dye Tyramide
92208... 92228	CF® Dye & Biotin SE Protein Labeling Kits
92230... 92463	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
22013	Bovine Serum Albumin Fraction V
22014	Bovine Serum Albumin 30% Solution

Please visit our website at www.biotium.com to view our full selection of reactive CF® Dyes, labeled antibodies, Mix-n-Stain™ Antibody Labeling kits, and other CF® Dye conjugates.

Sephadex is a registered trademark of GE Healthcare.
Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

References

1. Bioconjugate Techniques (1st ed.) (1996); 2. Anal Biochem 185,131 (1990); 3. Anal Biochem 156, 220 (1986); 4. Biochim Biophys 200, 546 (1970); 5. Biochim Biophys 160, 272 (1968); 6. Nanotheranostics 2, 347 (2018); 7. Proc Natl Acad Sci USA 116, 9831 (2019).