Revised: June 29, 2020

Product Information

CF® Dye Amine

Unit Size: 1 mg

Catalog no.	Dye	Ex/Em (nm)	Extinction coefficient	MW
92035	CF®350	347/448	18,000	~537
92036	CF®405S	408/452	33,000	~725
92037	CF®488A	490/515	70,000	~704
92038	CF®555	555/565	150,000	~943
92039	CF®568	562/583	100,000	~756
92040	CF®594	593/614	115,000	~771
92041	CF®633	630/650	100,000	~863
92043	CF®640R	642/662	105,000	~1034
92042	CF®647	650/665	240,000	~1027
96010	CF®660R	663/682	100,000	~930
92102	CF®750	755/777	250,000	~3005
92065	CF®770	770/797	250,000	~3134

Storage and Handling

Store CF® dye amine at -20°C, protected from light. Product is stable for at least 12 months from date of receipt if stored as recommended. Stock solutions may be prepared in DMSO or dH_2O and can be stored at ≤ -20°C for at least 12 months.

Product Description

CF® dye amines can be conjugated to activated carboxylic acids in proteins or other molecules using carbodiimide chemistry. EDC (EDAC) (catalog no. 59002) may be used for direct coupling of carboxylic acids to primary amines using carbodiimide chemistry. Alternatively, EDC may be coupled with N-Hydroxysuccinimide (NHS) to prepare semi-stable NHS esters from carboxylate groups that can then be conjugated to primary amines. Our CF® amine derivatives are bright, photostable and water-soluble, making them an excellent choice for fluorescent labeling.

References

1) Hermanson, G. (1996). Bioconjugate Techniques (1st ed.); 2) Biochim. Biophys. 200(3), 546(1970); 3) Biochim. Biophys. 160(2). 272(1968); 4) Anal. Biochem. 185(1), 131(1990); 5) Anal. Biochem. 156(1), 220(1986); 6) Nanotheranostics. 2(4), 347(2018); 7) Proc Natl Acad Sci USA. 116(20), 9831(2019).

Experimental Protocols

Direct EDC Coupling Protocol for Labeling Proteins with CF® Dye Amines

EDC is a popular carbodiimide for conjugating 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. 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.[2-3] In addition, EDC may promote unwanted polymerization due to the presence of both amines and carboxylates on protein molecules. The following protocol has been adapted from literature for conjugating CF® dye amines to proteins using EDC coupling.[1,4,5] 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 (see related products)
- Reaction Buffer: 0.1 M MES (2-[*N*-morpholino]ethanesulfonic acid), pH 4.7-6
- Protein sample in Reaction Buffer
- EDC (EDAC) (catalog no. 59002)
- PBS buffer (pH 7.4) (catalog no. 22020)
- (Optional) Ultrafiltration Vial (see related products)
- Sephadex®; see Table 1 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 also be used. 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 nmole of protein total. Therefore add 100 nmole 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: 0.5-0.1 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 excluded from the column corresponds to the antibody conjugate.

   Note: See Table 1 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 small than the protein molecular weight.

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

Two-Stage EDC/Sulfo-NHS Coupling Protocol for Labeling Proteins with CF® Dye Amines

The following protocol is a modified two-step protocol which involves activation of carboxyl proteins with EDC/sulfo-NHS and subsequent conjugation with CF® dye amines.[1,4] Sulfo-NHS improves the EDC coupling efficiency by increasing the stability of the *O*-acylisourea intermediate, thereby extending the half-life of the activated carboxylate to hours. The protocol involves an acidic pH of activation which provides greater stability for the active ester intermediate. 2-mercaptoethanol is also 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 (see related products)
- Reaction Buffer: 0.05 M MES (2-[*N*-morpholino]ethanesulfonic acid), 0.5 M NaCl, pH 6
- Protein sample in Reaction Buffer
- EDC (EDAC) (catalog no. 59002)
- Sulfo-NHS (*N*-Hydroxysuccinimide)
- 2-mercaptoethanol
- PBS buffer (pH 7.4) (catalog no. 22020)
- (Optional) Hydroxylamine
- (Optional) Ultrafiltration Vial (see related products)
- (Optional) Sephadex® G-25 desalting column
- Sephadex®; see Table 1 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.
3. Allow reaction to incubate for 15 minutes at room temperature.
4. Add 2-mercaptoethanol to the a 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 5)
5. 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.
6. Add 10-fold molar excess of CF® Dye Amine dissolved in concentrated PBS (catalog no. 22020) or other non-amine buffer to increase pH above 7.0. For example, 200 uL of 50 uM protein in reaction buffer is 10 nmole of protein total. Therefore add 100 nmole of CF® Dye Amine (or 20 uL of 5 mM stock solution). Mix reaction well.

   Note: The increase in pH above 7.0 is require to initiate the active ester reaction.
7. Incubate reaction for at least 2 hours at room temperature in the dark.
8. 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.
9. 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 protein conjugate.

   Note: See Table 1 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.
10. Store conjugate in an appropriate buffer and temperature for the protein of interest, protected from light.

Table 1. CF® Dye Technical Data

Dye	Sephadex® media	A_{max} (nm)	A_{280}/A_{max} or C_f (protein)	Extinction coefficient (ε)	Optimal DOL (IgG)
CF®350	G-25	347	0.14	18,000	4-6
CF®405S	G-25	404	0.7	33,000	5-10
CF®405M	G-25	408	0.13	41,000	4-6
CF®405L	G-25	395	0.5	24,000	8-12
CF®430	G-25	426	0.044	40,000	5-8
CF®440	G-25	440	0.044	40,000	5-8
CF®450	G-25	450	0.2	40,000	5-8
CF®488A	G-25	490	0.1	70,000	7-9
CF®503R	G-25	503	0.09	90,000	4-10
CF®514	G-25	516	0.073	105,000	5-8
CF®532	G-25	527	0.06	96,000	4-7
CF®543	G-25	541	0.095	100,000	4-7
CF®550R	G-25	551	0.08	100,000	5-6
CF®555	G-25	555	0.08	150,000	4-5, 3-6 ok
CF®568	G-25	562	0.08	100,000	5-6
CF®570	G-25	568	0.1	150,000	5-6
CF®583	G-25	583	0.223	150,000	5-6
CF®594	G-25	593	0.08	115,000	4-7
CF®620R	G-25	617	0.45	115,000	5-6
CF®633	G-25	630	0.48	100,000	4-7
CF®640R	G-50	642	0.37	105,000	4-7
CF®647	G-25	650	0.03	240,000	4-5, 3-6 ok
CF®660C	G-75	667	0.08	200,000	3-6, 2-3 ok
CF®660R	G-25	663	0.51	100,000	4-7
CF®680	G-75	681	0.09	210,000	3-5, 2-3 ok
CF®680R	G-25	680	0.32	140,000	5-6
CF®700	G-75	695	0.06	240,000	3-6
CF®750	G-75	755	0.03	250,000	3-5, 2-3 ok
CF®770	G-75	770	0.06	220,000	3-5, 2-3 ok
CF®790	G-75	784	0.07	210,000	3-5
CF®800	G-75	797	0.08	210,000	3-5
CF®820	G-75	822	0.07	253,000	3-6

Related Products

Catalog number	Product
22004	Ultrafiltration Vial, 10K MWCO (5 per pack)
22018	Ultrafiltration Vial, 3K MWCO (5 per pack)
90082	DMSO, Anhydrous, 10 mL
59002	EDC (EDAC), 100 mg
22013	Bovine Serum Albumin, Fraction V, 50 g
22014	Bovine Serum Albumin, 30% Solution, 100 mL
22020	10X Phosphate Buffered Saline, 4 L
41024-4L	Water, Ultrapure Molecular Biology Grade, 4 L

Please visit www.biotium.com to view our full selection of CF® reactive dyes and labeling kits, CF® dye labeled antibodies and other conjugates, and more.