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Product Information

CF® Dye Succinimidyl Ester (SE)

Catalog no.	Dye	Unit size	Abs/Em (nm)	Extinction coefficient	MW (free acid form)
92109	CF®350	1 umol	347/448	18,000	~496
92110	CF®405S	1 umol	404/431	33,000	~1169
92111	CF®405M	1 umol	408/452	41,000	~503
92112	CF®405L	1 umol	395/545	24,000	~1573
92117	CF®430	1 umol	426/498	40,000	~429
92123	CF®440	1 umol	440/515	40,000	~716
96011	CF®450	1 umol	450/538	40,000	~689
92120	CF®488A	1 umol	490/515	70,000	~914
92103	CF®514	1 umol	516/548	105,000	~1216
92104	CF®532	1 umol	527/558	96,000	~685
92105	CF®543	1 umol	541/560	100,000	~870
92130	CF®555	1 umol	555/565	150,000	~959
92131	CF®568	1 umol	562/583	100,000	~714
96014	CF®570	1 umol	568/591	150,000	~2998
96016	CF®583	1 umol	583/606	150,000	~3127
92132	CF®594	1 umol	593/614	115,000	~729
92106	CF®620R	1 umol	617/639	115,000	~738
92133	CF®633	1 umol	630/650	100,000	~821
92108	CF®640R	1 umol	642/662	105,000	~832
92135	CF®647	1 umol	650/665	240,000	~1058
92137	CF®660C	1 umol	667/685	200,000	~3112
92134	CF®660R	1 umol	663/682	100,000	~888
92139	CF®680	1 umol	681/698	210,000	~3241
92107	CF®680R	1 umol	680/701	140,000	~912
96067	CF®700	1 umol	695/720	240,000	~3197
92142	CF®750	1 umol	755/777	250,000	~3060
92150	CF®770	1 umol	770/797	220,000	~3189
92155	CF®790	0.25 umol	784/806	210,000	~3318
92127	CF®800	0.25 umol	797/816	210,000	~3334
96068	CF®820	0.25 umol	822/835	253,000	~3434

Storage and Handling

Store desiccated at ≤ -20°C. Product is stable for at least 1 year from date of receipt when stored as directed.

Product Description

Succinimidyl Ester (SE or NHS ester) CF® dyes are amine-reactive forms of Biotium's bright and photostable CF® dyes. The succinimidyl ester group of the dye reacts with an amine group to form a stable amide linkage. CF® dyes are next-generation fluorescent dyes that have combined advantages in brightness, photostability, and water-solubility compared to other dyes such as Alexa Fluor®, DyLight®, Cy® Dyes or IRDyes®.

Biotium also offers CF® Dye SE Protein Labeling Kits, which contain everything required for labeling and purification of 3 x 1 mg IgG reactions. Our VivoBrite™ Rapid Antibody Labeling Kits for Small Animal Imaging allow you to label an antibody with one of our near-IR CF® dyes for *in vivo* imaging. We also offer Mix-n-Stain™ Antibody Labeling Kits, which allow you to label 5-100 ug of antibody in 30 minutes with no purification step.

Protocol for labeling IgG antibodies

The protocol below is a typical procedure for labeling IgG antibodies in bicarbonate buffer; 1 umol dye is sufficient to label 8-15 mg IgG; 0.25 umol dye is sufficient to label 2-3 mg IgG. The protocol may require modifications for labeling other proteins.

1. Materials required but not provided

- IgG: the IgG should be free of any amine-containing stabilizers, such as amino acids, Tris, BSA or gelatin, as these substances will also react with the dye. Small molecules like Tris or amino acids can be removed by dialyzing the antibody against PBS buffer, or using an ultrafiltration vial to exchange the buffer (see related products). The presence of azide does not affect the labeling reaction.
- Anhydrous DMSO (see related products)
- Sodium bicarbonate ($NaHCO_3$)
- Sephadex®; see Table 1 for the appropriate type of Sephadex® for each CF® dye
- PBS buffer (pH~7.4)
- Sodium azide (NaN_3)
- BSA (see related products)

2. Labeling procedure

2.1 Prepare antibody solution for labeling

Dissolve the antibody in 0.1 M sodium bicarbonate buffer (pH~8.3) at 2.5 mg/mL. If the IgG is already dissolved in a buffer such as PBS, the labeling solution can be prepared by adding one-tenth volume of 1 M sodium bicarbonate solution (pH 8.3) to the IgG solution for a final bicarbonate concentration of 0.1 M.

Note:
At about 2.5 mg/mL protein concentration, the labeling efficiency is generally around 35%. A protein concentration of less than 2.5 mg/mL is also suitable for labeling, although the labeling efficiency will be lower. A labeling efficiency of 20-30% can be expected with a protein concentration around 1 mg/mL. Even higher labeling efficiency is possible with protein concentration higher than 5 mg/mL. Because of variations in buffer and protein purity, accurate labeling efficiency can only be determined under your exact conditions. If the IgG solution is too dilute, it may be concentrated using an ultrafiltration vial with 10 kDa molecular weight cut-off (10K MWCO; see related products).

2.2 Prepare dye stock solution

Allow the vial of CF® dye SE to warm up to room temperature. Prepare a 10 mM dye stock solution. For 1 umol dye: add 100 uL anhydrous DMSO to the vial. For 0.25 umol dye: add 25 uL anhydrous DMSO to the vial. Vortex the vial briefly to fully dissolve the dye, followed by brief centrifugation to collect the dye at the bottom of the vial.

Notes:
1) If the labeling reaction is to be carried out with a small amount of protein, the dye stock solution may need to be more dilute for accurate pipetting.

2) Unused stock solution may be stored at -20°C, protected from light and moisture. If anhydrous DMSO is used for making the solution, the dye should be stable for at least one month.

3) Dye stock solution may also be prepared in dH_2O or aqueous buffer. However, because the dye will hydrolyze over time, aqueous stock solutions should be prepared immediately before the conjugation reaction and cannot be stored for later use.

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2.3 Carry out the labeling reaction

a) While stirring or vortexing the protein solution, add 15-25 uL of the 10 mM dye stock per mL of antibody solution in a dropwise fashion. These volumes correspond to dye/protein molar ratios between 9:1 to 15:1.

Note:
As stated in Step 2.1, 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. See Table 1 for the optimal degree of labeling or DOL (number of dyes conjugated to each protein) for each CF® dye. A DOL slightly above or below the optimal range will also produce good results.

b) Continue to stir or rock the reaction solution at room temperature for 1 hour, protected from light.

Note:
While the labeling reaction is underway, proceed to Step 2.4a to prepare a Sephadex® column. See Table 1 for the appropriate Sephadex® medium to use for each CF® dye.

2.4 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) Immediately load the reaction solution from Step 2.3b onto the column and elute the column with PBS buffer. The first band excluded from the column corresponds to the antibody conjugate.

Notes:
1) 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. 10K MWCO can be used for IgG; proteins with different molecular weights may require different MWCO.

2) If you choose not to separate the labeled antibody from the free dye immediately after the reaction, you may add 50 uL of 1 M lysine to stop the reaction.

3. Determination of degree of labeling (DOL)

3.1 Determine the protein concentration

The concentration of the antibody conjugate can be calculated from the formula:
$[\text{conjugate}] = \{[A_{280} - (A_{max} \times C_f)]/1.4\} \times \text{dilution factor}$

where [conjugate] is the concentration of the antibody conjugate collected from the column in mg/mL; "dilution factor" is the fold of dilution used for spectral measurement; A_{280} and A_{max} are the absorbance readings of the conjugate at 280 nm and the absorption maximum respectively; C_f is the absorbance correction factor; and the value 1.4 is the extinction coefficient of IgG in mL/mg. See Table 1 for the A_{max} and correction factor for each CF® dye.

Notes:
1) The protein solution eluted from the column may be too concentrated for accurate absorbance measurement and thus must be diluted to approximately ~0.1 mg/mL. The fold of dilution ("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.

2) If labeling a protein other than IgG, use the extinction coefficient for that specific protein.

3.2 Calculate the degree of labeling (DOL)

The DOL is calculated according to the formula:
$\text{DOL} = (A_{max} \times \text{Mwt} \times \text{dilution factor})/(\varepsilon \times [\text{conjugate}])$

where A_{max}, "dilution factor" and [conjugate] are as defined in Step 3.1, Mwt is the molecular weight of IgG (~150,000), and ε is the molar extinction coefficient of the dye (see Table 1). Table 1 lists the optimal range of DOL for each dye, although a DOL slightly above or below this range will also produce good results.

4. Storage and handling of labeled antibody

For long-term storage, we recommend adding 5-10 mg/mL BSA and 0.01-0.03% sodium azide to the conjugate solution to prevent denaturation and microbial growth. The conjugate solution should be stored at 4°C and protected from light. If glycerol is added to a final concentration of 50%, the conjugate can be stored at -20°C. Under these conditions, antibody conjugates are stable for a year or longer.

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®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®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
22013	Bovine Serum Albumin, Fraction V
22014	Bovine Serum Albumin, 30% solution
22020	10X Phosphate Buffered Saline
41024-4L	Water, Ultrapure Molecular Biology Grade

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.