Revised: July 16, 2020

Product Information

CF® Dye Succinimidyl Ester (SE)

Catalog no.	Dye	Unit size	Ex/Em (nm)	MW (free acid form)
92109	CF®350	1 umol	347/448	~496
92110	CF®405S	1 umol	404/431	~1169
92111	CF®405M	1 umol	408/452	~503
92112	CF®405L	1 umol	395/545	~1573
92117	CF®430	1 umol	426/498	~429
92123	CF®440	1 umol	440/515	~716
96011	CF®450	1 umol	450/538	~689
92120	CF®488A	1 umol	490/515	~914
96078	CF®503R	1 umol	503/532	~1100
92103	CF®514	1 umol	516/548	~1216
92104	CF®532	1 umol	527/558	~685
92105	CF®543	1 umol	541/560	~870
96073	CF®550R	1 umol	551/577	~686
92130	CF®555	1 umol	555/565	~959
92131	CF®568	1 umol	562/583	~714
96014	CF®570	1 umol	568/591	~2998
96016	CF®583	1 umol	583/606	~3127
96084	CF®583R	1 umol	586/609	~773
92132	CF®594	1 umol	593/614	~729
92106	CF®620R	1 umol	617/639	~738
92133	CF®633	1 umol	630/650	~821
92108	CF®640R	1 umol	642/662	~832
92135	CF®647	1 umol	650/665	~1058
92137	CF®660C	1 umol	667/685	~3112
92134	CF®660R	1 umol	663/682	~888
92139	CF®680	1 umol	681/698	~3241
92107	CF®680R	1 umol	680/701	~912
96067	CF®700	1 umol	695/720	~2474
92142	CF®750	1 umol	755/777	~3009
92150	CF®770	1 umol	770/797	~3138
92155	CF®790	0.25 umol	784/806	~3267
92127	CF®800	0.25 umol	797/816	~3334
96068	CF®820	0.25 umol	822/835	~2553

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®.

Labeling Protocol

The protocol below is a typical procedure for labeling IgG antibodies in bicarbonate buffer or IgM antibodies in PBS (pH~7.4). For labeling most other proteins or antibodies that are stable at pH 8.3, the IgG labeling protocol with the appropriate dye:protein ratio would be the most suitable. For IgG, 1 umol dye is sufficient to label 8-15 mg IgG; 0.25 umol dye is sufficient to label 2-3 mg IgG. The optimal dye amounts for labeling IgM or other proteins needs to be determined empirically.

1. Materials required but not provided

- IgG or IgM antibodies 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

For labeling IgG
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.

For labeling IgM
IgM antibodies are pH-sensitive and must be prepared in a neutral pH solution. Prepare your IgM antibody solution at 2.5 mg/mL to 5 mg/mL concentration in PBS (pH~7.4).

Note:
For IgG, the labeling efficiency is generally around 35% at 2.5 mg/mL protein concentration. 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 IgG concentration around 1 mg/mL. Even higher labeling efficiency is possible with an IgG concentration higher than 5 mg/mL. IgM labeling is much less efficient than IgG labeling because hydrolysis dominates the process at neutral pH. Because of variations in buffer and protein purity, accurate labeling efficiency can only be determined under your exact conditions. If the antibody 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) For labeling IgM antibodies, you may need to prepare a more concentrated SE stock solution; see Section 2.3.
2) 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.

3) 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.

4) 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.

2.3 Carry out the labeling reaction

For labeling IgG

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/IgG molar ratios between 9:1 to 15:1. Volume of dye added may need to be adjusted to achieve optimal DOL.

For labeling IgM

Labeling is less efficient for IgM antibodies because hydrolysis dominates over labeling at neutral pH. For this reason, the dye/IgM molar ratio needs to be on the order of 50:1 or 100:1. While stirring or vortexing the protein solution, add 70-140 uL of 10 mM dye stock per mL of antibody solution in a dropwise fasion. These values correspond to dye/IgM molar ratios between 50:1 to 100:1. The concentration of dye in the stock solution may be increased up to 20 mM if more dye is needed to achieve an optimal DOL.

Note:
If IgM labeling efficiency is poor, an overnight incubation at 4°C with a 30:1 dye/IgM molar ratio may reduce hydrolysis and improve labeling efficiency.

2.4 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 2.5a to prepare a Sephadex® column. 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. 10K MWCO can be used for IgG or IgM; proteins with different molecular weights may require different MWCO. 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.

2.5 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 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.

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:
$[\text{conjugate}] = \{[A_{280} - (A_{max} \times C_f)]/\varepsilon\} \times \text{dilution factor}$

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 ε is the extinction coefficient in mL/mg. The extinction coefficient for IgG and IgM is 1.4 and 1.18 respectively. See Table 1 for the A_{max} and correction factor for each CF® dye.

Notes:
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.

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) or IgM (~180,000), 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. If labeling a protein other than immunogloblulin, use the extinction coefficient for that specific protein.

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®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®583R	G-25	586	0.08	100,000	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.