Revised: December 19, 2023

Product Information

CF® Dye SE/TFP

Cat. No.	Dye	Unit size	Ex/Em (nm)	MW (free acid form)
92109	CF®350 SE	1 umol	355/450	~496
92110	CF®405S SE	1 umol	411/431	~1169
92111	CF®405M SE	1 umol	416/452	~503
92112	CF®405L SE	1 umol	413/547	~1573
97502	CF®410 SE	5 mg	404/455	~242
92117	CF®430 SE	1 umol	424/497	~429
92123	CF®440 SE	1 umol	433/512	~479
96011	CF®450 SE	1 umol	448/533	~689
92120	CF®488A SE	1 umol	490/516	~914
96078	CF®503R SE	1 umol	503/532	~1100
97500	CF®505 SE	1 umol	505/519	~587
92103	CF®514 SE	1 umol	516/549	~1216
92104	CF®532 SE	1 umol	531/552	~685
92105	CF®543 SE	1 umol	543/563	~887
96073	CF®550R SE	1 umol	551/577	~686
92130	CF®555 SE	1 umol	554/568	~959
92131	CF®568 SE	1 umol	562/584	~714
96014	CF®570 SE	1 umol	568/592	~2998
96016	CF®583 SE	1 umol	584/606	~3127
96084	CF®583R SE	1 umol	585/609	~773
92132	CF®594 SE	1 umol	593/615	~729
96092	CF®597R SE	1 umol	597/619	~800
92106	CF®620R SE	1 umol	620/643	~738
92133	CF®633 SE	1 umol	629/650	~821
92108	CF®640R SE	1 umol	642/663	~832
92135	CF®647 SE	1 umol	652/668	~985
92137	CF®660C SE	1 umol	667/685	~3024
92134	CF®660R SE	1 umol	662/682	~888
92139	CF®680 SE	1 umol	681/698	~3153
92107	CF®680R SE	1 umol	680/701	~912
96067	CF®700 SE	1 umol	696/721	~2474
92142	CF®750 SE	1 umol	755/779	~2921
92150	CF®770 SE	1 umol	770/797	~3091
92155	CF®790 SE	0.25 umol	783/808	~3179
92127	CF®800 SE	0.25 umol	797/817	~3334
96068	CF®820 SE	0.25 umol	822/835	~2711
96094	CF®850 TFP	0.25 umol	852/870	~2787
96095	CF®870 TFP	0.25 umol	876/896	~2773

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 addressed the limitations of other commonly used fluorescent dyes for improved compatibility and signal-to-noise.

CF® Dye TFP (tetrafluorophenyl) esters are more stable alternatives to succinimidyl ester (SE or NHS ester). CF® Dye TFP esters are available for CF®850 and CF®870, Biotium's industry-leading near-infrared dyes with emission above 850 nm.

Labeling Protocols

Considerations for protein labeling

The protocol provided 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 need to be determined empirically.

Considerations for nucleic acid labeling

CF® Dye Succinimidyl Esters can be used to label DNA modified with free amine groups, either by enzymatic incorporation of aminoallyl-modified nucleotides (see Related Products), or oligonucleotide synthesis with a terminal amino group. For oligonucleotide labeling, we recommend using an HPLC-purified oligo, with an amino group with a C6 linker at the 5' or 3' end. A method yielding the appropriate degree or purity should then be used to remove free dye after labeling, with common methods being ethanol precipitation, reverse phase HPLC, and cation exchange chromatography. Alternatively, an oligo synthesis company could perform this for you as a custom labeling.

Antibody Labeling Protocol

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)

1. Labeling procedure

1.1 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

Commonly used protocols for labeling IgM antibodies use buffer at neutral pH. Prepare your IgM antibody solution at 2.5 mg/mL to 5 mg/mL concentration in PBS (pH~7.4). Our Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits can also be used for fast and easy labeling of IgM (see Related Products)

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 labeling efficiency will be lower. A labeling efficiency of 20-30% can be expected with an IgG concentration around 1 mg/mL. 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).

1.2 Prepare dye stock solution

Allow the vial of CF® Dye ester (SE or TFP) 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:

a. For labeling IgM antibodies, you may need to prepare a more concentrated dye stock solution; see Section 1.3.

b. 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.

c. 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.

d. 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.

1.3 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 fashion. 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.

1.4 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 1.5a 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.

1.5 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 1.3 onto the column and elute the column with PBS buffer. The first band excluded from the column corresponds to the antibody conjugate.

2. Determination of degree of labeling (DOL)

2.1 Determine the protein concentration

The concentration of the antibody conjugate can be calculated from the formula:

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

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

Note: 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.

2.2 Calculate the degree of labeling (DOL)

The DOL is calculated according to the formula:

$$\text{DOL} = (A_{max} \times \text{Mwt} \times \text{dilution factor})/(\varepsilon_{dye} \times [\text{conjugate}])$$

Where A_{max}, "dilution factor" and [conjugate] are as defined in Step 2.1, Mwt is the molecular weight of IgG (~150,000) or IgM (~180,000), and ε_{dye} 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 immunoglobulin, use the extinction coefficient for that specific protein.

3. 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)	C_f		Extinction coefficient (ε)	Optimal DOL (IgG)
			A_{260}/A_{max}	A_{280}/A_{max}		
CF®350 SE	G-25	347	0.13	0.14	18,000	4-6
CF®405S SE	G-25	404	0.19	0.7	33,000	5-10
CF®405M SE	G-25	408	0.24	0.13	41,000	4-6
CF®405L SE	G-25	395	-	0.5	24,000	8-12
CF®410 SE	G-25	416	0.15	0.20	46,000	5-7
CF®430 SE	G-25	426	0.21	0.044	40,000	5-8
CF®440 SE	G-25	440	0.26	0.044	40,000	5-8
CF®450 SE	G-25	450	0.205	0.2	40,000	5-8
CF®488A SE	G-25	490	0.16	0.1	70,000	7-9
CF®503R SE	G-25	503	0.21	0.09	90,000	4-10
CF®505 SE	G-25	505	0.22	0.09	90,000	4-8
CF®514 SE	G-25	516	0.14	0.073	105,000	5-8
CF®532 SE	G-25	527	0.11	0.06	96,000	4-7
CF®543 SE	G-25	541	0.305	0.095	100,000	4-7
CF®550R SE	G-25	551	0.12	0.08	100,000	5-6
CF®555 SE	G-25	555	0.026	0.08	150,000	4-5, 3-6*
CF®568 SE	G-25	562	0.17	0.08	100,000	5-6
CF®570 SE	G-25	568	0.0998	0.1	150,000	5-6
CF®583 SE	G-25	583	0.139	0.223	150,000	5-6
CF®583R SE	G-25	586	0.19	0.08	100,000	5-6
CF®594 SE	G-25	593	0.24	0.08	115,000	4-7
CF®597R SE	G-25	597	0.25	0.08	100,000	5-6
CF®620R SE	G-25	617	0.28	0.45	115,000	5-6
CF®633 SE	G-25	630	0.25	0.48	100,000	4-7
CF®640R SE	G-50	642	0.23	0.44	105,000	4-7
CF®647 SE	G-25	650	0.01	0.03	240,000	4-5, 3-6*
CF®660C SE	G-75	667	0.05	0.08	200,000	3-6, 2-3*
CF®660R SE	G-25	663	0.20	0.51	100,000	4-7
CF®680 SE	G-75	681	0.06	0.09	210,000	3-5, 2-3*
CF®680R SE	G-25	680	0.155	0.32	140,000	5-6
CF®700 SE	G-75	695	0.055	0.06	240,000	3-6
CF®750 SE	G-75	755	0.01	0.03	250,000	3-5, 2-3*
CF®770 SE	G-75	770	0.041	0.06	220,000	3-5, 2-3*
CF®790 SE	G-75	784	0.104	0.07	210,000	3-5
CF®800 SE	G-75	797	0.09	0.08	210,000	3-5
CF®820 SE	G-75	822	0.0459	0.07	253,000	3-6
CF®850 TFP	G-75	852	-	0.06	240,000	3-6
CF®870 TFP	G-75	877	-	0.06	240,000	3-6

§ Sephadex recommendations are for antibody purification, not nucleic acid.
* Suitable, but suboptimal DOL.

Related Products

Cat. No.	Product
40020	5-Aminoallyl-dUTP
40021	5-Aminoallyl-UTP
92210-92226	CF® Dye & Biotin SE Protein Labeling Kits
92020...96079	CF® Dye Maleimides
92096-92099	CF® Dye MTS
92050-92059	CF® Dye Aminooxy
92151...96064	CF® Dye Hydrazides
92035-92102	CF® Dye Amine
92080..96000	CF® Dye Azide
92086...96006	CF® Dye Alkyne
92230...92433	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
92558...92575	Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits
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
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
22010	10X Fish Gelatin Blocking Agent
23002	EverBrite™ Mounting Medium with DAPI
23004	EverBrite™ Hardset Mounting Medium with DAPI
40060	RedDot™1 Far-Red Nuclear Stain
40061	RedDot™2 Far-Red Nuclear Stain
40083...41038	NucSpot® Nuclear Stains for Dead or Fixed Cells
40081-40082	NucSpot® Live Nuclear Stains
41024-4L	Water, Ultrapure Molecular Biology Grade

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.