March 20, 2024

Product Information

CF® Dye Maleimide

Cat. No.	Dye	Unit size	Ex/Em (nm)	MW (reactive form)
92020	CF®350	1 umol	355/450	~618
92030	CF®405S	1 umol	411/431	~626
92021	CF®405M	1 umol	416/452	~1293
92046	CF®405L	1 umol	413/547	~1695
92118	CF®430	1 umol	424/497	~551
92124	CF®440	1 umol	433/512	~839
96012	CF®450	1 umol	448/533	~810
92022	CF®488A	1 umol	490/516	~1036
96079	CF®503R	1 umol	503/532	~1222
92045	CF®532	1 umol	531/552	~808
92044	CF®543	1 umol	543/563	~1009
96074	CF®550R	1 umol	551/577	~808
92023	CF®555	1 umol	554/568	~932
92024	CF®568	1 umol	562/584	~836
96015	CF®570	1 umol	568/592	~3119
96017	CF®583	1 umol	584/606	~3248
96107	CF®583R	1 umol	585/609	~773
92025	CF®594	1 umol	593/615	~851
92033	CF®620R	1 umol	620/643	~860
92026	CF®633	1 umol	629/650	~945
92034	CF®640R	1 umol	642/663	~954
92027	CF®647	1 umol	652/668	~1106
92028	CF®660C	1 umol	667/685	~3234
92031	CF®660R	1 umol	662/682	~983
92029	CF®680	1 umol	681/698	~3363
92032	CF®680R	1 umol	680/701	~1034
96062	CF®750	0.5 umol	755/779	~3043
96108	CF®790	0.25 umol	783/808	~3301
92128	CF®800	0.25 umol	797/817	~3456
96069	CF®820	0.25 umol	822/835	~2955

Storage and Handling

Store desiccated at ≤ -20°C. CF® Dye maleimides are guaranteed for at least 6 months from date of receipt when stored as recommended.

Product Description

CF® Dye maleimides are thiol-reactive forms of Biotium's bright and photostable CF® Dyes. Maleimide reacts with thiol groups to form thioether-coupled products. The reaction can take place at pH 7 in the presence of amines. At neutral pH, the maleimide group does not react with histidine or arginine.

CF® Dyes are next-generation fluorescent dyes that have combined advantages in brightness, photostability, and water-solubility when compared to other commercially available dyes.

Labeling Protocol

The protocol below is a typical procedure for labeling IgG antibodies. Protocols for labeling other thiol- containing molecules are similar, except for the purification procedures which may need to be modified. The labeling reaction may be scaled up or down for any amount of protein as long as the ratios of the reagents are maintained.

Materials required but not provided

- Anhydrous DMSO (see Related Products)
- 10-100 mM phosphate (e.g., PBS), Tris, or HEPES buffer with pH 7.0-7.5
- Sephadex®; see Table 1 for the appropriate type of Sephadex® for each CF® Dye
- (Optional) Tris-(2-carboxyethyl)phosphine (TCEP; see Related Products) for reducing disulfide bonds in proteins to produce free thiol groups.
- Sodium azide (NaN_3)
- BSA (see Related Products)

1. Labeling procedure

1.1 Prepare antibody solution for labeling

a. Dissolve the antibody at 50-100 uM (7.5-15 mg/mL for IgG) in any of the buffers listed in the Materials section at room temperature.

b. As an optional step, if you wish to free up more thiol groups from the disulfide bonds in the protein, you may add ~10-fold molar excess of TCEP at this stage. Incubate the reaction solution for ~30 minutes. The reduction reaction and the subsequent labeling reaction are best carried out in the presence of an inert gas (N_2 or Ar) to prevent re-formation of disulfide bonds.

1.2 Prepare dye stock solution

Allow the vial of CF® Dye maleimide 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. For 0.5 umol dye: add 50 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. 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.

b. 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.

1.3 Carry out the labeling reaction

a. While stirring or vortexing the protein solution, add a volume of dye stock solution to result in a dye:protein molar ratio of 10-20. For example, for IgG at 50 uM, you would add dye to a final concentration of 0.5-1 mM.

b. Continue to stir or rock the reaction solution at room temperature for 2 hours or at 4°C overnight, protected from light.

Note: While the labeling reaction is underway, proceed to Step 1.4a to prepare a Sephadex® column. See Table 1 for the appropriate Sephadex® medium to use for each CF® Dye.

1.4 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. Load the reaction solution from Step 1.3b onto the column and elute the column with PBS buffer. The first band excluded from the column corresponds to the antibody conjugate.

Note: 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. Determination of degree of labeling (DOL)

2.1 Determine the protein concentration

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

where [conjugate] is the concentration of the antibody conjugate collected from the column; "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:

a. 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.

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

2.2 Calculate the degree of labeling (DOL)

The DOL is calculated according to the formula:
DOL = (A_{max} x Mwt x dilution factor)/(ε x [conjugate])

where A_{max}, "dilution factor" and [conjugate] are as defined in Step 2.1, Mwt is the molecular weight of IgG (~150,000), and ε is the molar extinction coefficient of the dye (see Table 1).

3. Storage and handling of labeled antibody

For long-term storage, we recommend that BSA and sodium azide be added to the conjugate solution to final concentrations of 5-10 mg/mL and 0.01-0.03%, respectively, 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)
			A_{260}/A_{max}	A_{280}/A_{max}	
CF®350	G-25	355	0.13	0.14	18,000
CF®405S	G-25	411	0.19	0.7	33,000
CF®405M	G-25	416	0.24	0.13	41,000
CF®405L	G-25	413	--	0.5	24,000
CF®430	G-25	424	0.21	0.044	40,000
CF®440	G-25	433	0.26	0.044	40,000
CF®450	G-25	448	0.205	0.2	40,000
CF®488A	G-25	490	0.16	0.1	70,000
CF®503R	G-25	503	0.21	0.09	90,000
CF®532	G-25	531	0.11	0.06	96,000
CF®543	G-25	543	0.305	0.095	100,000
CF®550R	G-25	551	0.12	0.08	100,000
CF®555	G-25	554	0.026	0.08	150,000
CF®568	G-25	562	0.17	0.08	100,000
CF®570	G-75	568	0.0998	0.1	150,000
CF®583	G-75	584	0.139	0.223	150,000
CF®583R	G-25	586	0.19	0.08	100,000
CF®594	G-25	593	0.24	0.08	115,000
CF®620R	G-25	620	0.28	0.45	115,000
CF®633	G-25	629	0.25	0.48	100,000
CF®640R	G-25	642	0.23	0.37	105,000
CF®647	G-25	652	0.01	0.03	240,000
CF®660C	G-75	667	0.05	0.08	200,000
CF®660R	G-25	662	0.20	0.51	100,000
CF®680	G-75	681	0.06	0.09	210,000
CF®680R	G-25	680	0.155	0.32	140,000
CF®750	G-75	755	0.01	0.03	250,000
CF®790	G-75	784	0.104	0.07	210,000
CF®800	G-75	797	0.09	0.08	210,000
CF®820	G-75	822	0.0459	0.07	253,000

§ Sephadex recommendations are for antibody purification, not nucleic acids or other proteins.

Related Products

Cat. No.	Product
40020	5-Aminoallyl-dUTP
40021	5-Aminoallyl-UTP
92210-92226	CF® Dye & Biotin SE Protein Labeling Kits
97502...92120	CF® Dye SE/TFP
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 www.biotium.com to view our full selection of CF® reactive dyes and labeling kits, CF® Dye labeled antibodies and other conjugates, and more.