

# Product Information

## CF® Dye Maleimide

Catalog no.	Dye	Unit size	Ex/Em (nm)	Extinction coefficient	MW
92020	CF@350	1 umol	347/448	18,000	~618
92030	CF@405S	1 umol	404/431	33,000	~626
92021	CF@405M	1 umol	408/452	41,000	~1293
92046	CF@405L	1 umol	395/545	24,000	~1695
92118	CF@430	1 umol	426/498	40,000	~551
92124	CF@440	1 umol	440/515	40,000	~839
96012	CF@450	1 umol	450/538	40,000	~810
92022	CF@488A	1 umol	490/515	70,000	~1036
96079	CF@503R	1 umol	503/532	90,000	~1222
92045	CF@532	1 umol	527/558	96,000	~808
92044	CF@543	1 umol	541/560	100,000	~1009
96074	CF@550R	1 umol	551/577	100,000	~808
92023	CF@555	1 umol	555/565	150,000	~932
92024	CF@568	1 umol	562/583	100,000	~836
96015	CF@570	1 umol	568/591	150,000	~3119
96017	CF@583	1 umol	583/606	150,000	~3248
92025	CF@594	1 umol	593/614	115,000	~851
92033	CF@620R	1 umol	617/639	115,000	~860
92026	CF@633	1 umol	630/650	100,000	~945
92034	CF@640R	1 umol	642/662	105,000	~954
92027	CF@647	1 umol	650/665	240,000	~1106
92028	CF@660C	1 umol	667/685	200,000	~3234
92031	CF@660R	1 umol	663/682	100,000	~983
92029	CF@680	1 umol	681/698	210,000	~3363
92032	CF@680R	1 umol	680/701	140,000	~1034
96062	CF@750	0.5 umol	755/777	250,000	~3043
92128	CF@800	0.25 umol	797/816	210,000	~3456
96069	CF@820	0.25 umol	822/835	253,000	~2955

### Storage and Handling

Store desiccated at  $\leq -20^{\circ}\text{C}$ . 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 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. 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 ( $\text{NaN}_3$ )
- BSA (see Related Products)

#### 1. Labeling procedure

##### 1.1 Prepare antibody solution for labeling

- Dissolve the antibody at 50-100  $\mu\text{M}$  (7.5-15 mg/mL for IgG) in any of the buffers listed in the Materials section at room temperature.
- As an optional step, if you wish to free up more thiol groups from the disulfide bonds in the protein, you may add  $\sim 10$ -fold molar excess of TCEP at this stage. Incubate the reaction solution for  $\sim 30$  minutes. The reduction reaction and the subsequent labeling reaction are best carried out in the presence of an inert gas ( $\text{N}_2$  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  $\mu\text{L}$  anhydrous DMSO to the vial. For 0.25 umol dye: add 25  $\mu\text{L}$  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:

- 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.
- Unused stock solution may be stored at  $-20^{\circ}\text{C}$ , 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

- 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  $\mu\text{M}$ , you would add dye to a final concentration of 0.5-1 mM.
- Continue to stir or rock the reaction solution at room temperature for 2 hours or at  $4^{\circ}\text{C}$  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

- Prepare a Sephadex® column (10 mm x 300 mm) equilibrated in PBS buffer (pH~7.4).
- 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:  

$$[\text{conjugate}] \text{ (mg/mL)} = \{[A_{280} - (A_{\text{max}} \times C_i)] / 1.4\} \times \text{dilution factor}$$

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  $A_{\text{max}}$  are the absorbance readings of the conjugate at 280 nm and the absorption maximum respectively;  $C_i$  is the absorbance correction factor; and the value 1.4 is the extinction coefficient of IgG in mL/mg. See Table 1 for the  $A_{\text{max}}$  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.
- 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:

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

where  $A_{\text{max}}$ , "dilution factor" and [conjugate] are as defined in Step 2.1, Mwt is the molecular weight of IgG (~150,000), and  $\epsilon$  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	$A_{\text{max}}$ (nm)	$A_{280}/A_{\text{max}}$ or $C_i$ (protein)	Extinction coefficient ( $\epsilon$ )
CF®350	G-25	347	0.14	18,000
CF®405S	G-25	404	0.7	33,000
CF®405M	G-25	408	0.13	41,000
CF®405L	G-25	395	0.5	24,000
CF®430	G-25	426	0.044	40,000
CF®440	G-25	440	0.044	40,000
CF®450	G-25	450	0.2	40,000
CF®488A	G-25	490	0.1	70,000
CF®503R	G-25	503	0.09	90,000
CF®532	G-25	527	0.06	96,000
CF®543	G-25	541	0.095	100,000
CF®550R	G-25	551	0.08	100,000
CF®555	G-25	555	0.08	150,000
CF®568	G-25	562	0.08	100,000
CF®570	G-25	568	0.1	150,000
CF®583	G-25	583	0.223	150,000
CF®594	G-25	593	0.08	115,000
CF®620R	G-25	617	0.45	115,000
CF®633	G-25	630	0.48	100,000
CF®640R	G-50	642	0.37	105,000
CF®647	G-25	650	0.03	240,000
CF®660C	G-75	667	0.08	200,000
CF®660R	G-25	663	0.51	100,000
CF®680	G-75	681	0.09	210,000
CF®680R	G-25	680	0.32	140,000
CF®750	G-75	755	0.03	250,000
CF®800	G-75	797	0.08	210,000
CF®820	G-75	822	0.07	253,000

## Related Products

Catalog number	Product
22004	Ultrafiltration vial, 10K MWCO (5 per pack)
22018	Ultrafiltration vial, 3K MWCO (5 per pack)
90082	DMSO, Anhydrous
91049	TCEP
22013	Bovine Serum Albumin, Fraction V
22014	Bovine Serum Albumin, 30% solution
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super <sup>HT</sup> Pap Pen 2.5 mm tip, ~400 uses
22006	Super <sup>HT</sup> Pap Pen 4 mm tip, ~800 uses

Please visit [www.biotium.com](http://www.biotium.com) to view our full selection of CF® reactive dyes and labeling kits, CF® Dye labeled antibodies and other conjugates, and more.

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