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Product Information

CF® Dye & Biotin SE Protein Labeling Kits

Size: 3 labelings (up to 1 mg protein each) per kit

Kit Components

Material	Quantity
CF® Dye SE or Biotin (Component A)	3 vials
DMSO, anhydrous (99953)	1 X 150 uL
Sodium bicarbonate solution, 1 M, pH 8.3 (99954)	1 X 1 mL
1X PBS, pH 7.4 (99955)	1 X 50 mL
Ultrafiltration vial, 10K MWCO (99956)	3 vials
Reaction vial (99957)	3 vials
Storage vial (99958)	3 vials

Storage and Handling

Store at 4°C. Stable for at least 3 months from date of receipt when stored as recommended. Stable for at least 6 months from date of receipt if the reactive dye vials (component A) are stored separately at -20°C.

Technical Summary

Kit Cat. No.	CF® Dye	Abs_{max} (nm)	Em_{max} (nm)	Extinction coefficient	MW (free acid form)
92224	Biotin	N/A	N/A	N/A	~341
92210	CF®350	347	448	18,000	~496
92211	CF®405S	404	431	33,000	~1169
92212	CF®405M	408	452	41,000	~503
92228	CF®405L	395	545	24,000	~1573
92213	CF®488A	490	515	70,000	~914
92208	CF®532	527	558	96,000	~685
92209	CF®543	541	560	100,000	~870
92214	CF®555	555	565	150,000	~901
92215	CF®568	562	583	100,000	~714
92216	CF®594	593	614	115,000	~729
92217	CF®633	630	650	100,000	~821
92225	CF®640R	642	662	105,000	~832
92218	CF®647	650	665	240,000	~1058
92219	CF®660C	667	685	200,000	~3112
92223	CF®660R	663	682	100,000	~888
92220	CF®680	681	698	210,000	~3241
92226	CF®680R	680	701	140,000	~912
92221	CF®750	755	777	250,000	~3009
92222	CF®770	770	797	220,000	~3138

Product Description

This kit provides a convenient way to label antibodies with an outstanding fluorescent CF® dye or biotin. The kit contains CF® dye succinimidyl ester (SE), or biotin SE, and everything else you need for carrying out the labeling reaction and purifying the labeled product. The dye is supplied in three vials, each of which is sufficient for labeling 1 mg of an IgG antibody. Following the labeling reaction, unconjugated dye is conveniently and rapidly removed by ultrafiltration using the ultrafiltration vials provided. Typical yield for preparing CF® dye antibody conjugates using this protocol is 80-90%.

Protocol for Labeling IgG Antibodies

The protocol below is for labeling 1 mg of an IgG antibody. The procedure may be scaled up or down for any amount of protein as long as the ratios of the reagents are maintained. Note: the kits are not recommended for labeling IgM antibodies, because the alkaline pH at which labeling is carried out may cause denaturation of IgM. The kit can be used to label non-IgG proteins. The ratio of dye stock solution to protein amount may require optimization for different proteins.

Depending on the protein molecular weight, the dye removal method may need to be modified; to remove free dye by ultrafiltration, the protein should be at least 3X larger than the molecular weight cut-off of the ultrafiltration membrane. For ultrafiltration of proteins with molecular weight of 10-30 kDa, we recommend using ultrafiltration vials with 3 kDa molecular weight cut-off (22018).

Note: Warm all reagents to room temperature before use.

1. **Prepare the Antibody for Labeling**

   If the antibody is already in solution at ≥1 mg/mL in PBS or a similar buffer free of any amine-containing chemicals or preservatives, such as Tris, ammonium or free amino acids (such as glycine), proceed to step 2. However, if any amine-containing chemical is present, perform ultrafiltration using the filtration vial (99956) provided in the kit. Sodium azide does not affect the labeling.

   The ultrafiltration column has a molecular weight cut-off of 10,000. Molecules smaller than 10 kDa will flow through the membrane, and molecules larger than 10 kDa, including IgG antibodies, will be retained on the upper surface of the membrane (Figure 1). Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody.
   Note: Repeated filtration of large sample volumes (~500 uL) can lead to membrane failure. We therefore recommend keeping sample volumes at or below 350 uL.

   Ultrafiltration Vial Capacities:
   Maximum Sample Volume: 500 uL
   Filtrate Receiver Volume: 500 uL
   Hold-up Volume (Membrane/Support): < 5 uL

Ultrafiltration Protocol

1.1 Add an appropriate amount of antibody to the membrane of the ultrafiltration vial, being careful not to touch the membrane. Spin the solution at 14,000 X g in a microcentrifuge for one minute. Check to see how much liquid has filtered into the filtrate collection tube (lower chamber). Repeat the centrifugation until all of the liquid has filtered into the collection tube. Discard the liquid in the collection tube.

1.2 For antibody concentration, proceed to Step 1.3. For clean-up, add an equal volume of 1X PBS to the membrane. Spin the vial at 14,000 X g until the liquid has filtered into the filtrate receiving tube.

1.3 To resuspend and recover the antibody, add 400 uL PBS to the sample reservoir and carefully pipet up and down over the upper surface of the membrane.

1.4 Transfer the recovered antibody solution to a fresh microcentrifuge tube. Add 500 uL PBS to make a final volume of 900 uL.

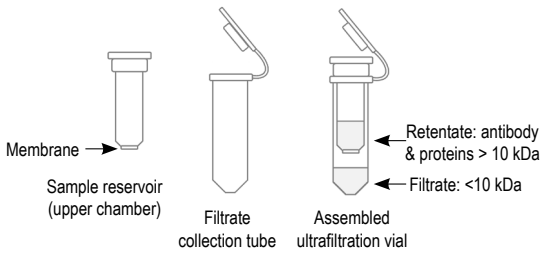

Figure 1. Ultrafiltration vial components.

1.5 **Important:** Save the ultrafiltration vial to reuse in the antibody purification step (Step 3). Ultrafiltration vials also can be purchased separately (22004).

2. **Carry Out the Labeling Reaction**

2.1 Add 100 uL of 1M sodium bicarbonate pH 8.3 (99954) to the 900 uL antibody solution from step 1.4. If you did not perform ultrafiltration, adjust the antibody concentration to 1-2 mg/mL in 1X PBS (99955) and add 1/10 volume of 1M sodium bicarbonate pH 8.3.

Note: Labeling efficiency varies with antibody concentration. In general, the higher the antibody concentration, the higher the labeling efficiency of the dye. This protocol is designed to result in optimal DOL (see Table 1) when antibody concentration is at 1-2 mg/mL.

2.2 Allow a vial of CF® dye or biotin SE to warm up to room temperature, and then add 25 uL anhydrous DMSO (99953) to the dye vial. Vortex to dissolve the dye and then centrifuge briefly to collect the dye solution at bottom of the vial.

2.3 Transfer the dye stock from Step 2.2 to the antibody solution prepared in Step 2.1 and mix well. Protect the antibody/dye solution from light by wrapping the vial in aluminum foil and incubate the reaction for 1 hour at room temperature with gentle rocking.

3. **Purify the Labeled Antibody**

3.1 Transfer up to 350 uL of the reaction solution from Step 2.3 to the upper chamber of a filtration vial (99956). Centrifuge the vial at 14,000 xg until nearly all of the liquid is in the collection tube below (5-10 min). Empty the collection tube, which contains unconjugated free dye.

Caution! Avoid touching the membrane of the filtration vial during liquid transfer using a pipet. Any damage to the membrane may result in loss of antibody.

3.2 Repeat step 3.1 until all of the antibody/dye solution has been centrifuged. A small amount of 1X PBS (99955) may be used to rinse the reaction vial and complete the solution transfer.

3.3 Wash the labeled antibody. Add 300 uL 1X PBS (99955) to the upper chamber of the filtration vial and flick the vial gently to fully dissolve the labeled antibody. Centrifuge as above.

3.4 Repeat step 3.3 two more times. By the third ultrafiltration, the fluorescent color of the solution in the collection tube (visualized using a UV lamp) should be very light, indicating nearly complete removal of the free dye from the labeled antibody.

3.5 Add an appropriate amount of 1X PBS to the filter sample reservoir to resuspend the antibody at the desired concentration. Place the antibody solution in the provided storage vial (99958). Typical recovery is 80-90%.

4. **Determine the Degree of Labeling** (DOL; number of dye molecules per antibody)

4.1 Measure the absorbance of the antibody solution prepared in Step 3.5 at 280 nm and at the absorption maximum of the CF® dye used (see Table 1).

Note: The antibody solution is typically too concentrated for accurate absorbance measurement and should be diluted to approximately ~0.1 mg/mL. For example, if 1 mg of antibody was labeled, and the antibody was resuspended in ~0.5 mL PBS in step 3.5, the antibody concentration would be roughly 2 mg/mL. Therefore, you would need to perform a 1:20 dilution (i.e., dilution factor = 20) for spectral measurement.

4.2 Calculate the final concentration of the antibody conjugate ([conjugate]) using the formula:

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

In the above formula:

- "dilution factor" is the fold of dilution used for spectral measurement (see note below);
- A_{280} and A_{max} are the absorbance readings of the conjugate at 280 nm and the absorption maximum for the CF® dye, respectively; C_f is the absorbance correction factor (See Table 1, below).
- The value 1.4 is the extinction coefficient of whole (H+L) IgG.

Note: Proteins other than whole IgG may have very different extinction coefficients. For non-IgG proteins, substitute the extinction coefficient of the specific protein you are labeling.

4.3 Calculate the DOL according to the formula:

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

In the above formula:

- A_{max}, dilution factor and [conjugate] are as defined in Step 4.2;
- Mwt is the molecular weight of IgG: ~150,000;
- ε is the molar extinction coefficient of the CF® dye (see Table 1).

The optimal DOL for CF® dyes in antibody labeling is listed in Table 1 below. A slight deviation from above optimal DOL range should still produce acceptable results.

5. **Storage**

Store the labeled antibody at 4°C and protect from light.

Note: Long term storage of antibodies at less than 0.1 mg/mL may result in adsorption of antibody to plastic vials and degradation of antibody.
Note: Sodium azide can be added as a preservative at a final concentration of 2 mM.

Table 1. CF® Dye Technical Data

CF® Dye	Abs_{max} (nm)	Extinction coefficient (ε)	A_{280}/A_{max} or C_f (protein)	Optimal DOL (IgG)
CF®350	347	18,000	0.14	4-6
CF®405S	404	33,000	0.7	5-10
CF®405M	408	41,000	0.13	4-6
CF®405L	395	24,000	0.5	8-12
CF®488A	490	70,000	0.1	7-9
CF®532	527	96,000	0.06	4-7
CF®543	541	100,000	0.095	4-7
CF®555	555	150,000	0.08	4-5, 3-6 ok
CF®568	562	100,000	0.08	5-6
CF®594	593	115,000	0.08	4-7
CF®633	630	100,000	0.48	4-7
CF®640R	642	105,000	0.37	4-7
CF®647	650	240,000	0.03	4-5, 3-6 ok
CF®660C	667	200,000	0.08	3-6, 2-3 ok
CF®660R	663	100,000	0.51	4-7
CF®680	681	210,000	0.09	3-5, 2-3 ok
CF®680R	680	140,000	0.32	5-6
CF®750	755	250,000	0.03	3-5, 2-3 ok
CF®770	770	220,000	0.06	3-5, 2-3 ok

Related Products

Catalog #	Product Name
22004	Ultrafiltration vial, 10K MWCO (5 pack)
22018	Ultrafiltration vial, 3K MWCO (5 pack)
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23013	TrueBlack® WB Blocking Buffer Kit
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
40083	NucSpot® 470 Nuclear Stain, 1000X in DMSO
22020	10X Phosphate Buffered Saline (PBS) (4L Cubitainer®)

Please visit www.biotium.com to view our full selection of CF® reactive dyes, Mix-n-Stain antibody labeling kits, VivoBrite antibody labeling kits for small animal imaging, a wide selection of CF® dye conjugates and other innovative products for life science research.