Product Information

CF® Dye & Biotin SE Protein Labeling Kits

Kit Contents

Component	Size
CF® Dye SE or Biotin SE (Component A)	3 vials
99953: DMSO, Anhydrous	1 x 150 uL
99954: 1 M Sodium Bicarbonate Solution, pH 8.3	1 x 1 mL
99955: 1X PBS, pH 7.4	1 x 50 mL
99956: Ultrafiltration Vial, 10K MWCO	3 vials
99957: Reaction Vial	3 vials
99958: Storage Vial	3 vials

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

Storage and Handling

Store CF® Dye SE or Biotin SE (Component A) at -20°C, desiccated and protected from light. Store sodium bicarbonate and PBS at 4°C. Store DMSO at room temperature, 4°C, or -20°C, desiccated and protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Product Description

CF® Dye & Biotin SE Protein Labeling Kits provide a convenient way to label proteins with an outstanding fluorescent CF® Dye or biotin. The kits contain CF® Dye succinimidyl ester (SE), or biotin SE, and everything else you need to carry out the labeling reaction and purify the labeled product. The dye is supplied in three vials, each of which is sufficient for labeling 1 mg of IgG antibody or an equivalent molar amount of protein. Following the labeling reaction, unconjugated dye is conveniently and rapidly removed by ultrafiltration using the ultrafiltration vials provided.

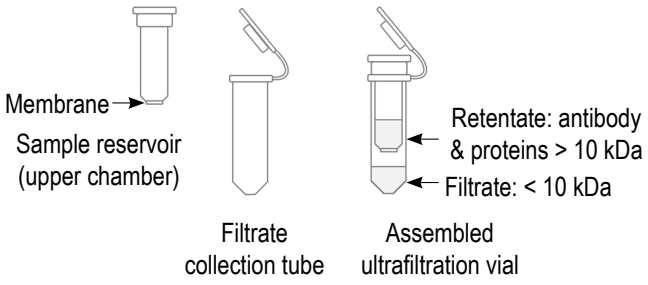

Figure 1. Ultrafiltration vial components.

Kit Compatibility and Protocol Selection

The following are general considerations for kit compatibility and selecting the appropriate protocol for labeling.

- These kits are recommended for labeling proteins, including antibodies, followed with a purification step to remove free dye after labeling. The protocol includes instructions for determining the degree of labeling, which may require optimization for your antibody or protein. For fast and simple labeling antibodies, nanobodies, or small ligands without a purification step, we recommend using one of our Mix-n-Stain™ Labeling Kits (see Related Products).
- The protein should be free of 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 protein against 1X PBS, or using an ultrafiltration vial to exchange the buffer. Dialysis or ultrafiltration will not remove proteins like BSA or gelatin, and if present, the antibody or protein must be purified to remove other proteins before labeling.
- Sodium azide does not affect the labeling reaction.
- The concentration of the protein should be 6-15 uM. This corresponds to 1-2 mg/mL of IgG antibody. Because of variations in buffer and protein purity, accurate labeling efficiency can only be determined under your exact conditions. Our Molarity Calculator can be used to calculate the molarity of the antibody or protein.
- Concentrate the protein, if necessary. While we have included an ultrafiltration vial with a 10 kDa cut-off, the protein should be at least 3X larger than the molecular weight cut-off of the ultrafiltration membrane. For ultrafiltration of proteins with a molecular weight of 10-30 kDa, we recommend using ultrafiltration vials with 3 kDa molecular weight cut-off (Cat. No. 22018).
- To accurately calculate the concentration and degree of labeling of your protein, you will need to know the protein extinction coefficient (ε). Protein extinction coefficients are traditionally reported as ε%, or the absorbance of a 10 mg/mL solution. Because protein concentration is usually measured in mg/mL (or 0.1% w/v), for calculations it is convenient to convert ε% to ε(0.1%). To convert ε% to ε(0.1%), multiply ε% by 0.1. For IgG, ε% is 14, so ε(0.1%) is 1.4.
- Most protein extinction coefficients range from ε% 4 to 24, corresponding to ε(0.1%) of 0.4 to 2.4. If you do not know the extinction coefficient for the protein to be labeled, it can be calculated from the protein sequence using the Expasy ProtParam online protein analysis tool. To roughly estimate protein concentration, you can use ε(0.1%) of 1.

Labeling Protocol

The protocol below is based on a standard labeling of an IgG antibody at 1 mg/mL (6.67 uM). The protocol may need to be optimized for other proteins of interest. The procedure may be scaled up or down for any amount of protein as long as the ratios of the reagents are maintained.

Note: Warm all reagents to room temperature before use.

1. Prepare the protein for labeling

If the protein is at 6-15 uM 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. If the concentration is < 6 uM or any amine-containing chemical is present, perform ultrafiltration using the ultrafiltration vial provided in the kit. Sodium azide does not affect the labeling.

The ultrafiltration column has a molecular weight cut-off of 10 kDa. Molecules smaller than 10 kDa will flow through the membrane, and molecules larger than 10 kDa 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 protein. For ultrafiltration of proteins with a molecular weight of 10-30 kDa, we recommend using ultrafiltration vials with 3 kDa molecular weight cut-off (Cat. No. 22018).

Note: Repeated filtration of large sample volumes (~500 uL) can lead to membrane failure. Therefore, we recommend keeping sample volumes at or below 350 uL.

Ultrafiltration vial capacities:
- Maximum sample volume: 500 uL (see Note above)
- Filtrate receiver volume: 500 uL
- Hold-up volume (membrane/support): < 5 uL

1.1 Add an appropriate amount of protein to the membrane of the ultrafiltration vial, being careful not to touch the membrane. Spin the solution at 14,000 x g for 1 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.

Note: We recommend saving the filtrate solution after steps 1.1 and 1.2, so you can recover your protein in case of membrane failure during centrifugation.

1.2 To concentrate the protein, 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 collection tube.

1.3 To resuspend and recover the protein, add 400 uL of 1X PBS to the sample reservoir and carefully pipette up and down over the upper surface of the membrane.

1.4 Transfer the recovered protein solution to a fresh microcentrifuge tube. Add 500 uL of 1X PBS to make a final volume of 900 uL.

Important: Save the ultrafiltration vial to reuse in the purification step (step 3). Ultrafiltration vials can also be purchased separately (Cat. No. 22004).

2. Carry out the labeling reaction

2.1 Add 100 uL of 1 M sodium bicarbonate pH 8.3 (Cat. No. 99954) to the 900 uL protein solution from step 1.4. If you did not perform ultrafiltration, adjust the protein concentration to 6-15 uM in 1X PBS and add 1/10 volume of 1 M sodium bicarbonate pH 8.3.

Note: Labeling efficiency varies with protein concentration. In general, the higher the protein concentration, the higher the labeling efficiency.

2.2 Bring a vial of CF® Dye or Biotin SE to room temperature, then add 25 uL of anhydrous DMSO to the dye vial. Vortex to dissolve the dye and then centrifuge briefly to collect the dye solution at the bottom of the vial.

Note: Dye stock solution may be prepared in water or aqueous buffer, but 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 Add 25 uL of the dye stock from step 2.2 for every 1 mL of protein solution prepared in step 2.1 and mix well.

Notes:

a. The dye:protein volumes correspond to molar ratios between 9:1 and 15:1. This ratio of dye to protein usually results in ~4-6 dye molecules per protein molecule after the reaction is complete. The dye:protein ratio may need to be higher for a more dilute protein solutions.

b. Unused dye stock solution reconstituted in DMSO may be stored at -20°C, desiccated and protected from light. The reconstituted dye is stable for at least one month.

2.4 Protect the protein/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 protein

3.1 Transfer up to 350 uL of solution from step 2.4 to the upper chamber of the ultrafiltration vial. Centrifuge at 14,000 x g until all of the liquid is in the collection tube (5-10 minutes). Empty the collection tube containing unconjugated free dye.

Caution! Avoid touching the membrane of the ultrafiltration vial with a pipette tip. Any damage to the membrane may result in loss of protein.

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

3.3 Wash the labeled protein by adding 300 uL of 1X PBS to the upper chamber of the ultrafiltration vial and flick the vial gently to fully dissolve the labeled protein. Centrifuge as above in step 3.1.

3.4 Repeat step 3.3 two more times. 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 protein.

3.5 Add an appropriate amount of 1X PBS to the filter sample reservoir to resuspend the protein at the desired concentration. Place the protein solution in the provided storage vial (Cat. No. 99958).

4. Determine the degree of labeling

4.1 Measure the absorbance of the protein solution prepared in step 3.5 at 280 nm and at the absorption maximum of the CF® Dye used. See the technical datasheet for the absorption maximum of each CF® Dye.

Note: The protein solution is typically too concentrated for accurate absorbance measurement and should be diluted to ~0.1 mg/mL. For example, if 1 mg of protein was labeled and the protein was resuspended in ~0.5 mL PBS in step 3.5, the protein concentration would be ~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 protein conjugate using the formula:

$$[\text{conjugate}] = \{[A_{280} - (A_{max} \times C_f)] / \varepsilon(0.1\%)\} \times \text{dilution factor}$$

In the above formula:

- [conjugate] is the concentration in mg/mL of the protein conjugate collected from the column.
- A_{280} and A_{max} are the absorbance readings of the conjugate at 280 nm and the absorption maximum for the CF® Dye, respectively. See the technical datasheet for the A_{max} of each CF® Dye.
- C_f is the absorbance correction factor. See the technical datasheet for the correction factor of each CF® Dye.
- ε(0.1%) is the molar extinction coefficient of the protein, calculated by multiplying ε% by 0.1.
- "dilution factor" is the fold of dilution used for spectral measurement.

4.3 Calculate the DOL according to the formula:

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

In the above formula:

- A_{max}, dilution factor, and [conjugate] are as defined in step 4.2.
- MW is the molecular weight of the protein in g/mol.
- ε is the molar extinction coefficient of the CF® Dye. See the technical datasheet for the extinction coefficient of each CF® Dye.

5. Storage

Store the conjugate using the optimal storage conditions for the protein. For IgG conjugates, store the labeled protein at 4°C, protected from light. For long-term storage, we recommend adding 5-10 mg/mL of BSA and 0.01-0.03% of sodium azide to the conjugate solution to prevent denaturation and microbial growth.

Notes:

a. Long-term storage of proteins at less than 0.1 mg/mL may result in adsorption of protein to plastic vials and degradation of the protein.

b. If glycerol is added to a final concentration of 50%, the conjugate can be stored at -20°C and will be stable for at least one year.

Related Products

Cat. No.	Product
22004	Ultrafiltration Vials, 10K MWCO
22018	Ultrafiltration Vials, 3K MWCO
92230...92584	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
92558-92575	Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits
92500-92515	Mix-n-Stain™ Nanobody Labeling Kits
92549...92557	Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits
92350...92364	Mix-n-Stain™ CF® Dye Small Ligand Labeling Kits
92103...97502	CF® Dye SE/TFP Ester
40083...41040	NucSpot® Nuclear Stains
30131-30135	CytoLiner™ Fixed Cell Membrane Stains
00095-00099	ActinBrite™ High Affinity Phalloidin Conjugates
30071	AccuOrange™ Protein Quantitation Kit
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23013	TrueBlack® WB Blocking Buffer Kit
23007, 23011	TrueBlack® Lipofuscin Autofluorescence Quencher
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23016	EverBrite™ Hardset with NucSpot® 640
23017	EverBrite TrueBlack® Hardset Mounting Medium
23018	EverBrite TrueBlack® Hardset Mounting Medium with DAPI
23019	EverBrite TrueBlack® Hardset Mounting Medium with NucSpot® 640
23005	CoverGrip™ Coverslip Sealant

Please visit our website at www.biotium.com to view our full selection of fluorescent CF® Dye antibody conjugates and reactive dyes, fluorescent probes, cellular stains, and reagents for immunofluorescence microscopy and flow cytometry.

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