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Product Information

VivoBrite™ Rapid Antibody Labeling Kits for Small Animal *In Vivo* Imaging, CF® Dye SE

Catalog Numbers

92160: CF®680
92161: CF®750
92162: CF®770
92163: CF®790

Components

Material	Quantity
CF® Dye, SE (Component A)	3 X 0.1 umole
DMSO, anhydrous (#99953)	150 uL
Sodium bicarbonate solution, 1 M, pH 8.3 (#99954)	1 mL
1X PBS, pH 7.4 (#99955)	50 mL
Ultrafiltration vial, 10K MWCO (#99956)	3 X 1
Reaction vial (#99957)	3 X 1
Storage vial, sterile (#99958)	3 X 1
Syringe, 1 mL (#99959)	3 X 1
Syringe filter, 4 mm, 0.2 um (#99960)	3 X 1

Storage and Handling

Stored desiccated at 4°C. Product is stable for at least 3 months from date of receipt when stored as recommended. If the reactive dye (Component A) is stored separately at -20°C, the shelf-life of the kit can be extended to at least 6 months from date of receipt.

Spectral Properties

Cat. No.	CF® Dye	Abs/Em maxima (nm)	Extinction coefficient (ε)	A_{280}/A_{max} (correction factor)
92160	CF®680	681/698	~210,000	0.09
92161	CF®750	755/777	~250,000	0.03
92162	CF®770	770/797	~220,000	0.06
92163	CF®790	784/806	~210,000	0.07

Product Application

VivoBrite™ Rapid Antibody Labeling kits are designed for preparing fluorescently labeled antibodies for in vivo near-infrared (near-IR) fluorescence imaging in small animals. Each kit contains one of our superior near-IR CF® dyes and everything else you need for carrying out the labeling reaction and purifying the labeled product. (For more details on near-IR CF® dyes, see the near-IR CF® dye flyer at the Biotium website.)

The reactive dye has a succinimidyl ester group, which reacts with an amine group of the protein (i.e., lysine side-chain amine) to form a stable amide linkage. The dye is supplied in three separate vials, each containing sufficient dye for labeling 1-2 mg of IgG antibody. Following the labeling reaction, unconjugated dye is conveniently and rapidly removed using the provided ultrafiltration microcentrifuge vials.

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: Warm all reagents to room temperature before use.

1. **Prepare the Antibody for Labeling**

For labeling, the antibody should be at ≥1 mg/mL in PBS or similar buffer that is free of amine-containing chemicals. If the buffer contains amines such as Tris, ammonium, or amino acids, these must be removed or they will interfere with the labeling reaction. Small molecule amines can be removed by dialysis against PBS using a micro-dialysis device (not provided), or by ultrafiltration using a membrane filtration vial provided in the kit. Additional ultrafiltration vials can be purchased separately (cat. no. 22004). Sodium azide will not affect the labeling; note that sodium azide will be removed by ultrafiltration along with free dye in step 3.

If the antibody contains stabilizer proteins, such as gelatin or BSA, these will not be removed by dialysis or ultrafiltration, and the antibody must be purified using a commercial kit or standard protocols (not provided) before labeling.

To remove amine contaminants by ultrafiltration:

The ultrafiltration column membrane has a molecular weight cut-off of 10,000. Therefore, 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. 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 (see note above)
Final Concentrate Volume: 15 uL
Filtrate Receiver Volume: 500 uL
Hold-up Volume (Membrane/Support): < 5 uL

Procedure:

a) Load the antibody solution in the upper chamber of a filtration vial (cat. no. 99956) and centrifuge at 14,000 xg until nearly all the liquid is in the lower collection tube.

b) Empty the collection tube and add any additional antibody solution to the upper chamber. Repeat steps a & b until all the antibody solution has been centrifuged.

c) Resuspend the concentrated antibody in the upper chamber in 0.5 mL 1X PBS (cat. no. 99955) and centrifuge to complete the second round of ultrafiltration.

d) Repeat Step c.

e) Transfer the ultrafiltered antibody to the provided reaction vial (cat. no. 99957) in a total volume of ~0.9 mL 1X PBS.

f) Important: Save the used filtration vial for dye removal below.

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2. Carry Out the Labeling Reaction

a) If necessary, add 1X PBS so that the antibody concentration is ~1 mg/mL.

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 optimized for 1 mg/mL antibody concentration, at which the labeling efficiency of the dye can be expected to be ~30%. Thus, the degree of labeling (DOL) can be predicted using (n_d/n_p) x 30%, where n_d/n_p is the molar ratio of dye to antibody used in the labeling reaction.

b) Add 1/10 volume of 1M pH 8.3 sodium bicarbonate (cat. no. 99954) to the antibody solution.

c) Allow a vial of CF®680 SE (0.1 umole) to warm up to room temperature, and then add 25 uL anhydrous DMSO (cat. no. 99953). Vortex to dissolve the dye, then centrifuge briefly to collect the dye solution at the bottom of the vial.

d) Add ~12 uL of the dye stock from Step 2c to the antibody solution prepared in Step 2b (1 mg antibody at 1 mg/mL). Protect the antibody/dye solution from light by wrapping the vial in aluminum foil and incubate the reaction for 1 hour at room temperature on a slow rocker.

Note: At this dye-to-protein molar ratio (i.e., ~7.2), the degree of labeling (DOL) is expected to be 2~2.2 dye/protein. Animal studies using antibodies labeled with near-IR CF® dyes at a DOL of ~2 have produced excellent results. However, because our near-IR CF® dyes are engineered to be highly water soluble and less prone to fluorescence quenching, antibodies labeled with a higher DOL (i.e., 2-4) may produce even better in vivo results, although this has yet to be confirmed. If a higher DOL is desired, increase the dye-to-protein ratio. The optimal DOL will need to be determined empirically. Any left-over dye stock solution may be kept for later use. If stored desiccated at -20°C, the reactive dye solution is stable for at least one month.

3. Purify the Labeled Antibody

Note: See step 1 for a description of the ultrafiltration vial.
a) Transfer up to 0.5 mL of the reaction solution from Step 2c to the upper chamber of a filtration vial (cat. no. 99956). Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody.

b) Centrifuge the vial at 14,000 xg until nearly all of the liquid is in the collection tube below (5-10 minutes). A small amount of 1X PBS may be used to rinse the reaction vial and complete the solution transfer. Repeat until all of the antibody/dye solution has been centrifuged.

c) Empty the collection tube, which contains the unconjugated free dye. Add up to 0.3 mL 1X PBS (cat. no. 99955) to the upper chamber of the vial, which contains the labeled antibody. Flick or shake the tube gently to fully dissolve the labeled protein. Centrifuge to filter the antibody solution.

d) Repeat Step 3c two more times. By the third ultrafiltration, the color of the solution in the collection tube should be almost clear, indicating complete removal of the free dye from the labeled antibody. If some free dye is still detected in the third wash, one or two more washes may be necessary to completely remove the unconjugated dye.

e) Add an appropriate amount of 1X PBS to the upper chamber of the vial to achieve the desired antibody concentration and gently resuspend the labeled antibody.

f) Filter the labeled antibody solution from Step 3e using the provided syringe (cat. no. 99959) and syringe filter (cat. no. 99960) into a sterile storage vial (cat. no. 99958). Remove the cap from the syringe tip and remove the plunger. Firmly attach the filter to the end of the syringe with a twist. Add the labeled antibody carefully to the syringe with a pipette. Replace the plunger, and filter the antibody in a slow, dropwise fashion into the sterile collection vial.

Caution: Do not plunge the antibody solution through the filter too quickly or with too much force as the filter may disconnect from the syringe, causing liquid to spray out. When using syringe filters, we recommend wearing eye protection or a face shield, or performing filtration behind a hood screen.

Note: Typical yield for preparing CF® dye-labeled antibody using this protocol is 80-90%.

4. Determine the Degree of Labeling (DOL)

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

[conjugate] (mg/mL) = {[A_{280} - (A_{max} x CF)]/1.4} x dilution factor

In the above formula:

[conjugate] is the concentration (in mg/mL) of the antibody conjugate solution prepared in Step 3f;

"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 of the dye, respectively; CF is the absorbance correction factor (see spectral properties).

The value 1.4 is the extinction coefficient of whole (H+L) IgG. Proteins other than whole IgG may have very different extinction coefficients, and thus, the formula will have to be adjusted for accurate determination of DOL.

Note: the antibody solution prepared in Step 3f is typically too concentrated for accurate absorbance measurement and should be diluted to approximately ~0.1 mg/mL. For example, if the labeled antibody is in ~0.5 mL PBS, which would roughly give an antibody concentration of ~2 mg/mL, you will need to perform a 1:20 dilution (i.e., dilution factor = 20) for spectral measurement.

b) Calculate the DOL according to the formula:
DOL = (A_{max} x Mwt x dilution factor)/(ε x [conjugate])
In the above formula:
A_{max}, "dilution factor" and [conjugate] are as defined in Step 4a;
Mwt is the molecular weight of IgG: ~150,000;
and ε is the molar extinction coefficient (see spectral properties).

5. Storage of Labeled Antibody

a) Store the labeled antibody at 4°C and protect from light.
Note: Long term storage of antibodies at concentrations less than 0.1 mg/mL may result in binding of antibody to the vial and degradation of product.

Related Products

Cat. #	Product Name	Unit Size
22004	Ultrafiltration Vial, 10K MWCO	5 per pack
92139	CF®680, Succinimidyl Ester	1 umol
92142	CF®750, Succinimidyl Ester	1 umol
92150	CF®770, Succinimidyl Ester	1 umol
92155	CF®790, Succinimidyl Ester	0.25 umol
90082	DMSO, Anhydrous	10 mL
92220	CF®680 SE Protein Labeling Kit	3 x 1 mg labelings
92221	CF®750 SE Protein Labeling Kit	3 x 1 mg labelings
92222	CF®770 SE Protein Labeling Kit	3 x 1 mg labelings
92282	CF®680 Mix-n-Stain™ Antibody Labeling Kit	1 x (5-20 ug) labeling
92284	CF®750 Mix-n-Stain™ Antibody Labeling Kit	1 x (5-20 ug) labeling
92285	CF®770 Mix-n-Stain™ Antibody Labeling Kit	1 x (5-20 ug) labeling
92288	CF®790 Mix-n-Stain™ Antibody Labeling Kit	1 x (5-20 ug) labeling

Please visit **www.biotium.com** to view our full selection of products featuring bright and photostable CF® dyes, including secondary antibodies, streptavidin, Annexin V, and other conjugates, along with a wide selection of innovative products for life science research.

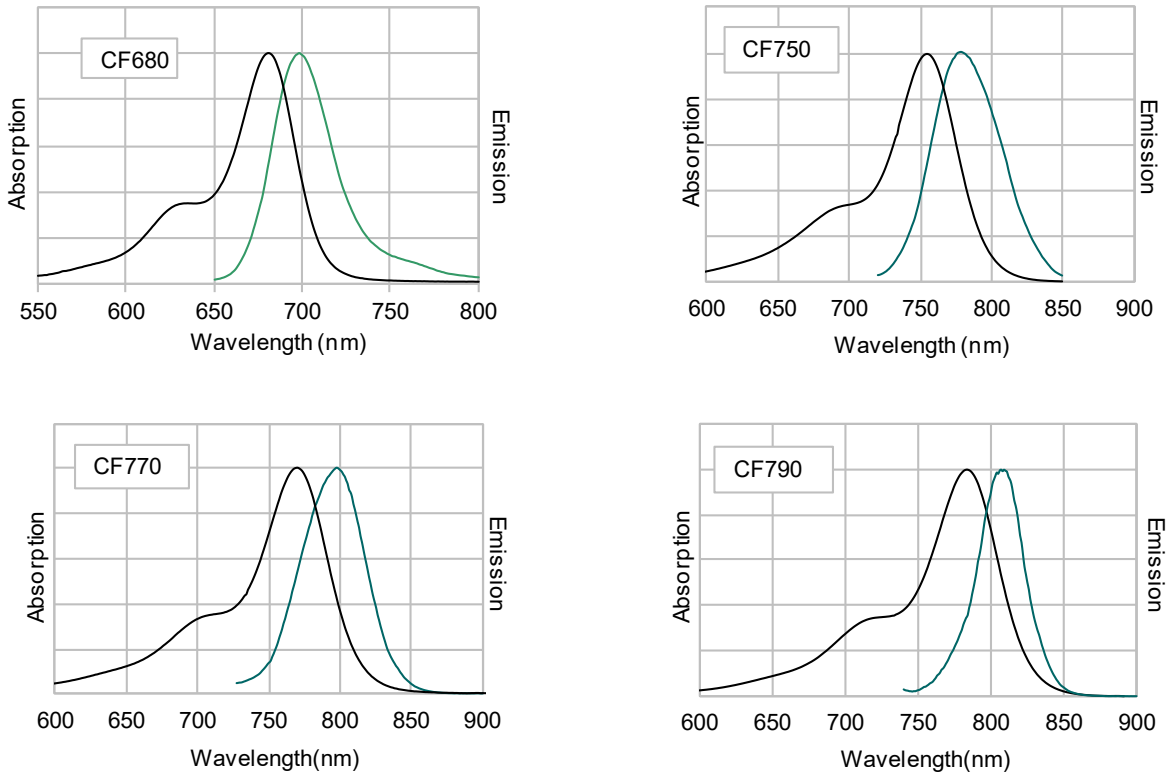

Figure 1. Absorption and emission spectra of VivoBrite™ dyes.