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Product Information

Mix-n-Stain™ RPE-CF® Dye Antibody Labeling Kits

Unit Size: 1 labeling reaction per kit

Product List

Label/Dye	Ex/Em (nm)	Emission filter	Labeling size/Catalog number		
			25-50 ug	50-100 ug	1 mg
RPE-CF®583R	496, 546, 586/609	PE-Texas Red®	92442	92443	---
RPE-CF®647T	496, 546, 555/665	PE-Cy®5	92340	92341	92346

Kit Contents

Component	25-50 ug labeling	50-100 ug labeling	1 mg labeling
Modified RPE-CF® Dye	1 vial Component A	1 vial Component A	1 vial Component A
Mix-n-Stain™ Reaction Buffer, 10X	15 uL 99951-1	30 uL 99951	1 mL 99951-1mL
Antibody Modifying Reagent	99807 1 vial	99807-1 1 vial	99807-2 1 vial
Mix-n-Stain™ Storage Buffer	150 uL 99952-1	300 uL 99952	1.8 mL 99952-3
Ultrafiltration vial	99956 2 vials	99956 2 vials	99956 2 vials

Materials required but not supplied: phosphate buffered saline (PBS).

Storage and Handling

Store at -20°C. Product is stable for at least 12 months from date of receipt when stored as recommended.

Product Description

Mix-n-Stain™ RPE-CF® Dye antibody labeling kits contain everything you need to rapidly conjugate an antibody to a tandem RPE-CF® Dye (RPE-CF®583R, RPE-CF®647T). These tandem dyes consist of R-phycoerythrin (R-PE) covalently attached to one of our bright and photostable CF® dyes as acceptors. CF®647T serves as a an alternative to Cy®5 and Alexa Fluor® 647. CF®583R is a rhodamine-based fluorescent dye that serves as an alternative to Texas Red®, CF®594, and Dazzle™ 594. CF®583R demonstrates excellent Förster resonance energy transfer (FRET) when paired with R-PE.

Choose the kit size corresponding to the amount of antibody you wish to label. Labeling takes 4 hours, wtih minimal hands-on time and no purification step after labeling. The resulting RPE-CF® Dye conjugate is stable for at least one month when stored at 4°C, or at least 3 months at -20°C in the storage buffer provided. Mix-n-Stain™ RPE-CF® Dye labeling can tolerate sodium azide. Two microcentrifuge ultrafiltration vials are provided in the kit. One vial is for rapid removal of incompatible buffer and small molecule antibody stabilizers such as glycerol before labeling (see Table 1). The other vial is used during the labeling protocol in Section B.

Biotium also offers Mix-n-Stain™ labeling kits for labeling antibodies with R-PE, APC, PerCP, and APC-CF®750 tandem dyes. Mix-n-Stain™ labeling kits are also available with our next-generation fluorescent CF® dyes, biotin, and FITC which allow rapid 30 minute conjugation without a purification step. Biotium's HRP, AP, and Glucose Oxidase antibody labeling kits can be used to conjugate antibodies to enzymes in about 3 hours.

Before you begin

Mix-n-Stain™ antibody labeling kits are optimized for labeling IgG antibodies. We do not recommend using them to label other proteins, because the degree of labeling may not be optimal. Mix-n-Stain™ labeling conditions may cause IgM antibodies to denature, therefore we do not recommend these kits for labeling IgM antibodies.

Check the compatibility of your antibody with the antibody compatibility guide below (Table 1). If your primary antibody is a commercial product, please contact the supplier to obtain the antibody concentration and formulation. Mix-n-Stain™ RPE-CF® Dye labeling can tolerate sodium azide. To remove glycerol and other small molecules, use the ultrafiltration vial provided in the kit to purify your antibody by following the steps in Section A.

If the antibody contains BSA or gelatin, or if the antibody is supplied as crude serum, ascites fluid, or hybridoma supernatant, purify the IgG prior to labeling using protein A purification or a commercial antibody clean-up kit, such as the Pierce Antibody Clean-Up Kit. Ultrafiltration will not remove stabilizer proteins from antibody solutions.

The optimal antibody concentration for labeling is 1-2 mg/mL. The ultrafiltration vial can be used to concentrate antibody solutions by following the steps in Section A. For quantitating antibodies of unknown concentration, Biotium offers the AccuOrange™ Protein Quantitation Kit (30071), a highly sensitive fluorescence-based protein assay.

Table 1. Mix-n-Stain™ RPE-CF® Dye Antibody Compatibility and Labeling Protocol Selection Guide

Component	Compatibility
Sodium Azide	Compatible, proceed to Section B
Glycerol	Perform ultrafiltration before labeling (Section A)
Tris	Perform ultrafiltration before labeling (Section A)
Glycine	Perform ultrafiltration before labeling (Section A)
Ascites fluid	Not compatible, purify IgG
Serum	Not compatible, purify IgG
Hybridoma supernatant	Not compatible, purify IgG

A. Ultrafiltration Protocol

Important: Two ultrafiltration vials are provided, one for use in Step A (if required) and one for use in Section B (Labeling Protocol). Before you begin, use Table 1 (Mix-n-Stain™ Antibody Compatibility and Labeling Protocol Selection Guide) to determine whether your antibody requires ultrafiltration before labeling. If necessary, contact the manufacturer of your antibody to find out the concentration of IgG and antibody stabilizers. If your antibody does not require ultrafiltration, proceed to the labeling protocol (Section B).

The ultrafiltration column membrane has a molecular weight cut-off of 10 kDa. 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 (Figure 1). Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody. Additional ultrafiltration vials also can be purchased separately (22004).

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 (350 uL recommended, see note above)
Final Concentrate Volume: 15 uL
Filtrate Receiver Volume: 500 uL
Hold-up Volume (Membrane/Support): < 5 uL

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.
2. For antibody concentration, proceed to Step 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.
3. Add an appropriate concentration of PBS to the membrane to obtain a final antibody concentration of 1-2 mg/mL. Carefully pipet the PBS up and down over the upper surface of the membrane to recover and resuspend the antibody.
4. Transfer the recovered antibody solution to a clean microtube.
5. Proceed to Section B.

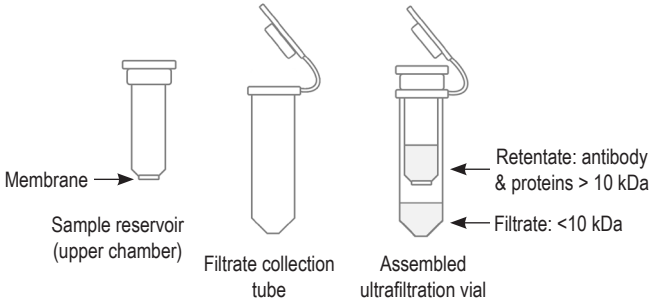

Figure 1. Ultrafiltration vial components.

B. Labeling Protocol

1. Use your antibody at 1-2 mg/mL for optimal conjugation. The ultrafiltration vial can be used to concentrate antibody solutions by following the steps in Section A. If your antibody is in lyophilized form, reconstitute in phosphate buffered saline (PBS).

   Note: The antibody can be dissolved in borate, carbonate or MOPS buffer. Antibody should be free of other proteins or preservatives such as BSA or gelatin.
2. Add 1/10 volume of Mix-n-Stain™ Reaction Buffer to your antibody solution (for example, add 1 uL Mix-n-Stain™ Reaction Buffer to 9 uL of antibody solution).
3. Add the above antibody solution to the vial of Antibody Modifying Reagent. Pipet the solution a few times up and down to mix with the Antibody Modifying Reagent. Briefly centrifuge the vial to collect the solution at the bottom of the vial.
4. Incubate the solution at room temperature for 1 hour.
5. Add the solution from step 4 to the membrane of a fresh ultrafiltration vial provided, being careful not to touch the membrane with the pipette tip. Add 200 uL phosphate buffered saline (PBS) to the membrane.

   Note: Two ultrafiltration vials are provided, one for use in Section A (only if required), and one for use in Section B.
6. Centrifuge the vial at 14,000 x g in a microcentrifuge for 5 minutes. The antibody will remain on the upper surface of the membrane. Discard the liquid in the collection tube.
7. Add an appropriate amount of PBS to the upper surface of the membrane to resuspend the antibody to a final concentration of 1 mg/mL based on the amount of antibody added to the reaction (for example, add 25 uL PBS if you are labeling 25 ug antibody or 100 uL PBS if you are labeling 100 ug antibody). Gently pipet the PBS up and down over the upper surface to the membrane to recover and resuspend the antibody.
8. Transfer the recovered antibody solution to the vial containing Modified RPE-CF® Dye (Component A) and vortex to dissolve the RPE-CF® Dye. Briefly centrifuge the vial to collect the solution at the bottom of the vial.

   Note: If the lyophilized RPE-CF® Dye is not totally dissolved, additional PBS can be added. A final concentration of 1 mg/mL of your IgG antibody is recommended (for example, if you start with 2 mg/mL, 25 uL IgG antibody solution, then add additional 25 uL 1X PBS).
9. Incubate the solution at room temperature in the dark for 1 hour.
10. For 25-50 ug and 50-100 ug labeling, transfer the entire volume of Storage Buffer to the reaction vial and vortex to mix. For 1 mg labeling, transfer the reaction solution to an appropriately sized vial, add the entire volume of Storage Buffer, and mix. Alternatively, you can add the storage buffer of your choice.
11. The RPE-CF® Dye conjugate is stable for at least one month when stored at 4°C, or at least 3 months at -20°C.

Related Products

Catalog number	Product
22004	Ultrafiltration vial, 10K MWCO
30071-T	AccuOrange™ Protein Quantitation Kit, trial size
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses
23006	Flow Cytometry Fixation/Permeabilization Kit
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22010	10% Fish Gelatin Blocking Buffer
22011	Fish Gelatin Powder
22014	30% Bovine Serum Albumin Solution
22002	Tween®-20

Other Mix-n-Stain™ Antibody Labeling Kits

Catalog number	Product
92298	Mix-n-Stain™ R-PE Antibody Labeling Kit, 1 X (25-50 ug) labeling
92299	Mix-n-Stain™ R-PE Antibody Labeling Kit, 1 X (50-100 ug) labeling
92306	Mix-n-Stain™ APC Antibody Labeling Kit, 1 X (25-50 ug) labeling
92307	Mix-n-Stain™ APC Antibody Labeling Kit, 1 X (50-100 ug) labeling
92308	Mix-n-Stain™ PerCP Antibody Labeling Kit, 1 X (25-50 ug) labeling
92309	Mix-n-Stain™ PerCP Antibody Labeling Kit, 1 X (50-100 ug) labeling
92310	Mix-n-Stain™ APC-CF®750T Antibody Labeling Kit, 1 X (25-50 ug) labeling
92311	Mix-n-Stain™ APC-CF®750T Antibody Labeling Kit, 1 X (50-100 ug) labeling

Please visit **www.biotium.com** to view our full selection of products including CF® dye Mix-n-Stain™ antibody labeling kits, secondary antibodies, streptavidin, anti-biotin, and anti-tag antibodies. Biotium also offers a variety of apoptosis and cell viability assays for flow cytometry analysis, including mitochondrial membrane potential dyes, fluorescent Annexin V conjugates, and NucView® 488 Caspase-3 Substrate for live cells.