Revised: March 18, 2021

Product Information

Mix-n-Stain™ APC-CF® Dye Antibody Labeling Kit

Unit Size: 1 labeling per kit

Label/Dye	Ex/Em (nm)	Emission filter	Labeling size/Catalog number	
			25-50 ug	50-100 ug
APC-CF®750T	496, 546, 650/780	APC-Cy®7	92310	92311
APC-CF®790T	496, 546, 784/806	APC-Cy®7*	92347	92348

*APC-CF®790T is intended for spectral flow cytometry. For conventional flow cytometry use APC-CF®750T.

Kit Contents

Component	25-50 ug labeling	50-100 ug labeling
Modified APC-CF® Dye	Component A 1 vial	Component A 1 vial
Mix-n-Stain™ Reaction Buffer	99951-1 1 vial	99951 1 vial
Antibody Modifying Reagent	99807 1 vial	99807-1 1 vial
Mix-n-Stain™ Storage Buffer	99952-1 1 vial	99952 1 vial
Ultrafiltration vial	99956 2 vials	99956 2 vials

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™ APC-CF® Dye Antibody Labeling Kits contain everything you need to rapidly conjugate an antibody to a tandem APC-CF® dye (APC-CF®750T, APC-CF®790T). These tandem dyes consist of allophycocyanin conjugated to one of our bright and photostable CF® dyes as acceptors. After labeling, the antibodies are ready to use for staining with no further purification step.

Choose the kit size corresponding to the amount of antibody you wish to label. After labeling and addition of our storage buffer, the APC-CF® dye conjugate is stable for at least one month when stored at 4°C, or at least 3 months at -20°C.

See Table 1 for Mix-n-Stain™ APC-CF® dye labeling compatibility with common buffer components. The labeling can tolerate sodium azide. Non-compatible, low molecular weight buffer components like glycerol and Tris can be removed by ultrafiltration. Two microcentrifuge ultrafiltration vials are provided in the kit. One vial is for rapid removal of incompatible buffer components; the other vial is used during the labeling protocol in Section B.

Biotium offers Mix-n-Stain™ kits to conjugate antibodies with R-PE, APC, PerCP, and tandem RPE-CF® dyes in about 4 hours. Mix-n-Stain™ 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 optimized. Mix-n-Stain™ labeling conditions may cause IgM antibodies to denature.

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. Ultrafiltration will not remove stabilizer proteins from antibody solutions.

The optimal antibody concentration for labeling is 1-2 mg/mL. One of the ultrafiltration vials provided in this kit 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™ APC-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 Section 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 10,000. 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 (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. Centrifuge 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. To concentrate the anitibody, 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 amount 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 microcentrifuge tube.
5. Proceed to Section B.

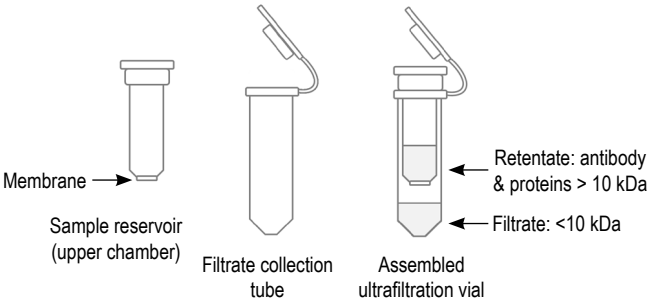

Figure 1. Ultrafiltration vial components.

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 PBS.

   Note: The antibody can be dissolved in borate, carbonate or MOPS buffer. Antibody should be free of other proteins 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, being careful not to touch the membrane with the pipette tip. Add 200 uL PBS to the membrane.

   Note: Two ultrafiltration vials are provided, one for use in Section A (only if required), and one for use in step 5.
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 solution from step 7 to the vial containing Modified APC-CF® dye (Component A) and vortex to dissolve the APC-CF® dye. If the lyophilized APC-CF® dye is not totally dissolved, additional amount of 1X 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). Vortex to completely dissolve the lyophilized APC-CF® dye. Briefly centrifuge the vial to collect the solution at the bottom of the vial.
9. Incubate the solution at room temperature in the dark for 1 hour.
10. Add an appropriate amount of Mix-n-Stain™ Storage Buffer for your desired concentration and vortex to mix. Transfer the entire solution to the vial you choose and the antibody is now ready for staining.
11. The APC-CF® dye conjugate is stable for at least one month when stored at 4°C, or at least 3 months at -20°C when using the supplied storage buffer.

Related Products

Catalog number	Product
22004	Ultrafiltration vial, 10K MWCO
30071	AccuOrange™ Protein Quantitation Kit
40017	Propidium Iodide
40085	NucSpot® Far-Red
40037	7-AAD
32010	Live-or-Dye NucFix™ Red
32016	Live-or-Dye™ Fixable Viability Sampler Kit, Standard
32017	Live-or-Dye™ Fixable Viability Samplet Kit, Spectral
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
92442	Mix-n-Stain™ RPE-CF®583R Antibody Labeling Kit, 1 X (25-50 ug) labeling
92340	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kit, 1 X (25-50 ug) labeling

Please visit **www.biotium.com** to view our full selection of products including Live-or-Dye™ Fixable Viability Staining 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.