Revised: June 1, 2017

Product Information

Mix-n-Stain™ PerCP Antibody Labeling Kit

Size: 1 labeling per kit

Storage: -20°C

Stability: Stable for at least 12 months from date of receipt when stored as recommended.

Components:

Component	92308 25-50 ug labeling	92309 50-100 ug labeling
Modified PerCP	92308A 1 vial	92309A 1 vial
Linking Agent	99997 1 vial	99997-1 1 vial
Conjugate Storage Buffer	99998-150uL 1 vial	99998-300uL 1 vial
Ultrafiltration vial	99956 2 vials	99956 2 vials

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

Product Application

Mix-n-Stain™ PerCP antibody labeling kits contain everything you need to rapidly conjugate an antibody to PerCP. Choose the kit size corresponding to the amount of antibody you wish to label. After labeling, the PerCP conjugate is stable for one month when stored at 4°C.

Mix-n-Stain PerCP labeling can tolerate Tris, glycine, and sodium azide. A microcentrifuge ultrafiltration vial is provided in the kit to rapidly remove incompatible small molecule antibody stabilizers such as glycerol before labeling (see Table 1). Labeling can be performed in the presence of up to four-fold excess of BSA or gelatin to IgG (by ug amount).

Biotium also offers Mix-n-Stain labeling kits for labeling antibodies with one of Biotium's next-generation fluorescent CF™ dyes, biotin, FITC in only 30 minutes without a purification step. Biotium's HRP antibody labeling kits can be used to conjugate antibodies to HRP in 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. Mix-n-Stain PerCP labeling can tolerate Tris, glycine, and sodium azide. To remove glycerol, use the ultrafiltration vial provided in the kit to purify your antibody by following the steps in Section A.

Antibodies can be labeled in the presence of up to 4-fold excess BSA or gelatin to IgG by weight. If the antibody contains more than 4-fold excess 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 Quantiation Kit (catalog no. 30071), a highly sensitive fluorescence-based protein assay.

Table 1. Mix-n-Stain™ PerCP Antibody Compatibility and Labeling Protocol Selection Guide

Component	Compatibility
Sodium Azide	Compatible, proceed to Section B
Glycerol	Perform ultrafiltration (Section A)
Tris	Compatible, proceed to Section B
Glycine	Compatible, proceed to Section B
BSA or gelatin	Up to 4X IgG (ug amount): Compatible, proceed to Section B More than 4X IgG (ug amount): Not compatible, purify IgG
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 10,000. 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 (cat. no. 22004).

Ultrafiltration Vial Capacities
Maximum Sample Volume: 500 µL
Final Concentrate Volume: 15 µL
Filtrate Receiver Volume: 500 µL
Hold-up Volume (Membrane/Support): < 5 µL

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 fresh microcentrifuge tube.

5. Proceed to Section B.

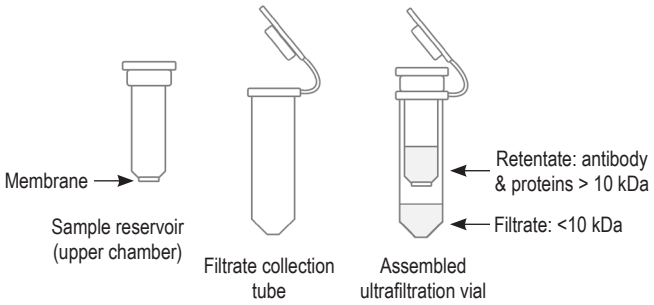

Figure 1. Ultrafiltration vial components.

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 phosphate buffered saline (PBS).

   Note: the antibody can be dissolved in Tris, borate, carbonate or MOPS buffer. Antibody should be free of other proteins or preservatives such as BSA or gelatin.

2. Add your antibody to the vial of Linking Agent (catalog no. 99997 or 99997-1, depending on kit size). Pipette the solution a few times up and down to mix with the Linking Agent.

3. Incubate the solution at room temperature for 30 minutes.

4. Add the solution from step 3 to the membrane of the provided ultrafiltration vial, 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.

5. 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.

6. Add an additional 200 uL PBS to the membrane. Centrifuge the vial at 14,000 x g 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 10 uL PBS if you are labeling 10 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 PerCP (92308A or 92309A, depending on kit size). Vortex to dissolve the lyophilized PerCP. Briefly centrifuge the vial to collect the solution at the bottom of the vial. Incubate the solution at room temperature in the dark for 3 to 4 hours.

9. Add Conjugate Storage Buffer (cat. 99998) For kit 92308 (25-50 ug labeling), add 100 uL storage buffer. For kit 92309 (50-100 ug labeling), add 200 uL storage buffer. Vortex to mix. The antibody is now ready for staining.

10. The PerCP conjugate is stable for at least a month when stored at -20°C.

Related Products

Catalog #	Product Name	Unit Size
22004	Ultrafiltration vial, 10K MWCO	5 per pack
30071-T	AccuOrange™ Protein Quantitation Kit, trial size	200 assays
23005	CoverGrip™ Coverslip Sealant	15 mL
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses	1 pen
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses	1 pen
30069	AccuEasy™ Flow Cytometry Kit	1 kit
23006	Flow Cytometry Fixation/Permeabilization Kit	50 tests
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
22017	Permeabilization and Blocking Buffer	100 mL
22010	10% Fish Gelatin Blocking Buffer	100 mL
22011	Fish Gelatin Powder	2 x 50 g
22014	30% Bovine Serum Albumin Solution	100 mL
22002	Tween®-20	50 mL

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.