Revised: March 24, 2021

Product Information

Mix-n-Stain™ R-PE Antibody Labeling Kit

Size: 1 or 3 labeling reactions per kit

Storage: -20°C

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

Kit Contents:

Component	92298 25-50 ug labeling	92299 50-100 ug labeling	92452 3x(25-50 ug) labeling
Modified R-PE	92298A 1 vial	92299A 1 vial	92298A 3 vials
Linking Agent	99997 1 vial	99997-1 1 vial	99997 3 vials
Conjugate Storage Buffer	99998-150uL 1 vial	99998-300uL 1 vial	99998-300uL 1 vial
Ultrafiltration vial	99956 2 vials	99956 2 vials	99956 6 vials

Product Application

Mix-n-Stain™ R-PE Antibody Labeling Kits contain everything you need to rapidly conjugate an antibody to R-phycoerythrin (R-PE). Choose the kit size corresponding to the amount of antibody you wish to label. After labeling, the R-PE conjugate is stable for at least one month when stored at 4°C, or at least 3 months at -20°C.

Mix-n-Stain™ R-PE 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).

See Related Products for APC, PerCP, and tandem dye labeling kits. Biotium also offers Mix-n-Stain™ labeling kits for labeling antibodies with one of Biotium's next-generation fluorescent CF® dyes, biotin, or FITC in only 30 minutes without a purification step. Mix-n-Stain™ enzyme labeling kits can be used to conjugate antibodies to HRP, AP, or glucose oxidase in about 3 hours.

Kit Compatibility and Protocol Selection

- Mix-n-Stain™ Antibody Labeling Kits are optimized for labeling IgG antibodies. The 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™ R-PE 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 Quantitation Kit (see Related Products), a highly sensitive fluorescence-based protein assay.

Table 1. Mix-n-Stain™ R-PE 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 for each reaction is provided for use in Section B (Labeling Protocol) and step A (if required). 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 vial 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 (see Related Products).

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

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 phosphate buffered saline (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 volume 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.

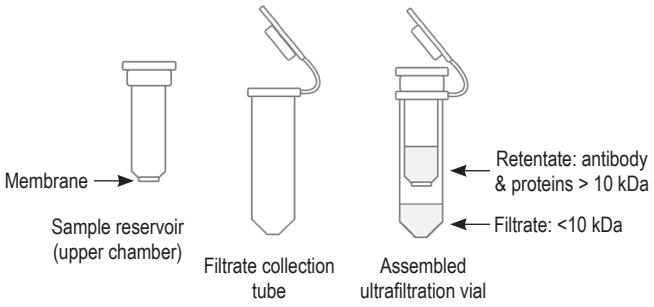

Figure 1. Ultrafiltration vial components.

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 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 (Cat 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 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 R-PE (92298A or 92299A, depending on kit size). Vortex to dissolve the lyophilized R-PE. 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 the appropriate amount of Conjugate Storage Buffer (Cat no. 99998) as shown in Table 2 below. Vortex to mix. The antibody is now ready for staining.

Table 2. Conjugate Storage Buffer Volume Required

Reaction Size	Conjugate Storage Buffer
25-50 ug	100 uL
50-100 ug	200 uL

10. The R-PE 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	AccuOrange™ Protein Quantitation Kit
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
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
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
92340	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kit, 1 X (25-50 ug) labeling
92341	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kit, 1 X (50-100 ug) labeling
92346	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kit, 1 X (1 mg) 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.