

Product Information

Mix-n-Stain™ Alkaline Phosphatase 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	92314 25-50 ug labeling	92315 50-100 ug labeling
Modified Alkaline Phosphatase	92314A 1 vial	92315A 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 1 vial	99956 1 vial

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

Product Application

Mix-n-Stain™ alkaline phosphatase antibody labeling kits contain everything you need to rapidly conjugate an antibody to alkaline phosphatase. Choose the kit size corresponding to the amount of antibody you wish to label. After labeling, the alkaline phosphatase 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™ alkaline phosphatase labeling can tolerate 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).

Biotium also offers Mix-n-Stain™ labeling kits for labeling antibodies with one of our next-generation fluorescent CF® dyes, biotin, FITC in only 30 minutes without a purification step. Biotium's HRP, glucose oxidase and fluorescent protein antibody labeling kits can be used to conjugate antibodies to HRP, glucose oxidase and various fluorescent proteins in 2-3 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. To remove glycerol and incompatible 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 (catalog no. 30071), a highly sensitive fluorescence-based protein assay.

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

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

A. Ultrafiltration Protocol

Important: An ultrafiltration vial is provided, for use in 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 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 clean microtube.
5. Proceed to Section B.

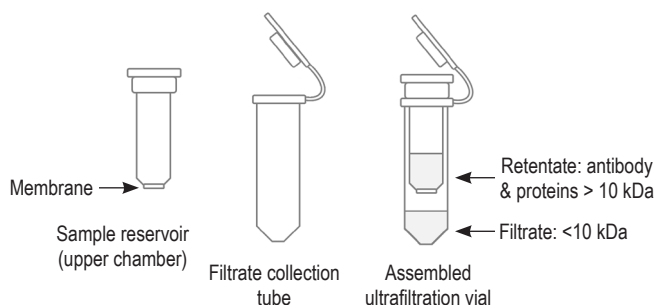


Figure 1. Ultrafiltration vial components.

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 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. Pipette 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. Transfer the solution from step 4 to the vial containing Modified Alkaline Phosphatase and vortex to dissolve the alkaline phosphatase. If the lyophilized alkaline phosphatase 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). Briefly centrifuge the vial to collect the solution at the bottom of the vial.
6. Incubate the solution at room temperature for 1 hour.
7. 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.
8. The alkaline phosphatase 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
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
10061	10-Acetyl-3,7-dihydroxyphenoxazine
30015	DAB Substrate Kit
10050	ABTS

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.

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