Revised: March 24, 2021

Product Information

Mix-n-Stain™ HRP 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	92300 10-20 ug labeling	92301 25-50 ug labeling	92302 50-100 ug labeling	92451 3x(10-20 ug) labeling
Modified HRP	92300A 1 vial	92301A 1 vial	92302A 1 vial	92300A 3 vials
Reaction Buffer	99994 25 uL	99994 25 uL	99994 25 uL	99994 25 uL
Reaction Enhancer	99995 1 vial	99995 1 vial	99995 1 vial	99995 1 vial
Storage Buffer	99996-70uL 70 uL	99996-150uL 150 uL	99996-300uL 300 uL	99996-300uL 300 uL
Ultrafiltration vial (MWCO=10K)	99956 1 vial	99956 1 vial	99956 1 vial	99956 3 vials

Product Application

Mix-n-Stain™ HRP Antibody Labeling Kits contain everything you need to rapidly conjugate an antibody to horseradish peroxidase (HRP). Choose the kit size corresponding to the amount of antibody you wish to label. After labeling, the HRP 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™ labeling can tolerate low levels of glycerol. A microcentrifuge ultrafiltration vial is provided in the kit to rapidly remove incompatible small molecule antibody stabilizers such as sodium azide, Tris, glycine, or excess 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 a Mix-n-Stain™ Maxi HRP Antibody Labeling Kit (1 mg) as well as alkaline phosphatase and glucose oxidase labeling kits. Biotium also offers Mix-n-Stain™ labeling kits for labeling antibodies with one of our next-generation fluorescent CF® dyes, biotin, FITC, PE, APC, PerCP, and tandem dyes.

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. An antibody solution free of stabilizers produces the best labeling results, however, low levels of glycerol in the antibody solution can be tolerated. To remove incompatible small molecules such as sodium azide, Tris, glycine or excess 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™ HRP Antibody Compatibility and Labeling Protocol Selection Guide

Component	Compatibility
Sodium Azide	Perform ultrafiltration (Section A)
Glycerol	Up to 10%: Compatible, proceed to Section B Greater than 10%: Perform ultrafiltration (Section A)
Tris	Perform ultrafiltration (Section A)
Glycine	Perform ultrafiltration (Section A)
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: 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.

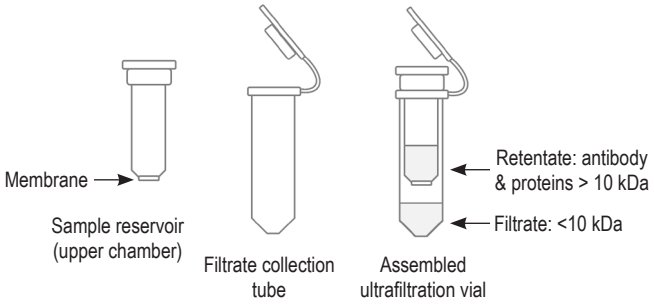

Figure 1. Ultrafiltration vial components.

B. Labeling Protocol

1. Use your antibody at 1-2 mg/mL for optimal conjugation. If your antibody is in lyophilized form, reconstitute in PBS.
2. Add 1/10 volume of Reaction Buffer (Cat no. 99994) to your antibody solution. For example, add 1 uL of reaction buffer to 10 uL antibody solution and mix well.
3. Add the solution from step 2 to the vial containing the modified HRP (92301A, 92302A, or 92303A, depending on kit size). Pipette the solution up and down to mix with the modified HRP.
4. Incubate the solution at room temperature in the dark for 3 hours.
5. Add 25 uL dH_2O to the vial containing the Reaction Enhancer (Cat no. 99995). Vortex to dissolve the enhancer.
6. Add the appropriate amount of reaction enhancer from step 5 to the solution from step 4 as shown in Table 2 below:

Table 2. Reaction Enhancer Volume Required

Reaction Size	Reaction Enhancer
10-20 ug	0.4 uL
25-50 ug	1 uL
50-100 ug	2 uL

7. Vortex to mix the solution and incubate room temperature in the dark for 15 minutes.
8. Add the appropriate amount of storage buffer (Cat no. 99996) to the solution from step 7 as shown in Table 3 below:

Table 3. Storage Buffer Volume Required

Reaction Size	Storage Buffer
10-20 ug	50 uL
25-50 ug	125 uL
50-100 ug	250 uL

9. Vortex to mix the solution and incubate room temperature in the dark for 15 minutes.
10. The antibody is now ready for staining. Antibody recovery is 100%. You can calculate the labeled antibody concentration by dividing the starting antibody amount by the total volume of solution after labeling. The labeled antibody is stable for at least one month when stored at 4°C, or at least 3 months at -20°C.

    Note: Buffers used for staining with HRP conjugates should not contain sodium azide, which inhibits HRP activity.

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
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

Other Mix-n-Stain™ Antibody Labeling Kits

Catalog number	Product
92437	Mix-n-Stain™ Maxi HRP Antibody Labeling Kit, 1 mg labeling
92314	Mix-n-Stain™ Alkaline Phosphatase Antibody Labeling Kit (25-50 ug) labeling
92315	Mix-n-Stain™ Alkaline Phosphatase Antibody Labeling Kit (50-100 ug) labeling
92312	Mix-n-Stain™ Glucose Oxidase Antibody Labeling Kit (25-50 ug) labeling
92313	Mix-n-Stain™ Glucose Oxidase Antibody Labeling Kit (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.