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Product Information

Mix-n-Stain™ HRP Antibody Labeling Kit

Size: 1 labeling per kit

Storage: -20ºC

Stability: Stable for at least 3 months from date of receipt when stored as recommended.

Components:

Component	Catalog# OKBE00064 10-20 ug labeling	Catalog# OKBE00065 25-50 ug labeling	Catalog# OKBE00066 50-100 ug labeling
Modified HRP	Part # 92300A 1 vial	Part # 92301A 1 vial	Part # 92302A 1 vial
Reaction buffer	Part # 99994 25 uL	Part # 99994 25 uL	Part # 99994 25 uL
Reaction enhancer	Part # 99995 1 vial	Part # 99995 1 vial	Part # 99995 1 vial
Storage buffer	Part # 99996-70uL 70 uL	Part # 99996-150uL 150 uL	Part # 99996-300uL 300 uL
Ultrafiltration vial (MWCO=10K)	Part # 99956 1 vial	Part # 99956 1 vial	Part # 99956 1 vial

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 one month when stored at 4ºC.

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).

Aviva also offers Mix-n-Stain labeling kits for labeling antibodies with one of Aviva's next-generation fluorescent CF™ dyes, biotin, or FITC.

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. 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 (note: stabilizer proteins will also be concentrated by the ultrafiltration vial).

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 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.

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.

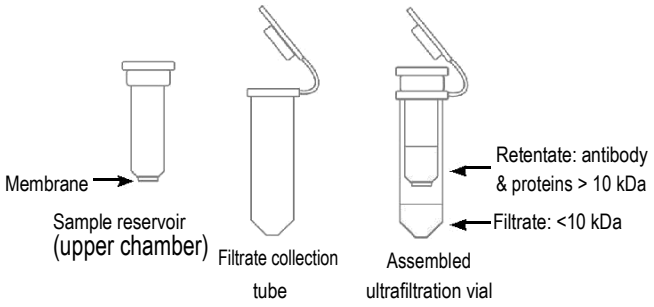

Figure 1. Ultrafiltration vial components.

Please visit **www.avivasysbio.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.

B. Labeling Protocol

1. Use your antibody at 1-2 mg/mL for optimal conjugation. If your antibody is in lyophilized form, reconstitute in phosphate buffered saline (PBS).
2. Add 1/10 volume of reaction buffer (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 (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 the table below:

	Catalog# OKBE00064	Catalog# OKBE00065	Catalog# OKBE00066
Kit size	10-20 ug labeling	25-50 ug labeling	50-100 ug labeling
Reaction enhancer	0.4 uL	1 uL	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 (99996-70uL, 99996-150uL, or 99996-300uL, depending on kit size) to the solution from step 7 as shown in the table below:

	Catalog# OKBE00064	Catalog# OKBE00065	Catalog# OKBE00066
Kit size	10-20 ug labeling	25-50 ug labeling	50-100 ug labeling
Storage buffer	50 uL	125 uL	250 uL

10. Vortex to mix the solution and incubate room temperature in the dark for 15 minutes.
11. 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 up to one month at 4ºC.
    Note: buffers used for staining with HRP conjugates should not contain sodium azide, which inhibits HRP activity.