

Product Information

Mix-n-Stain™ Maxi HRP Antibody Labeling Kit

Catalog Number: 92437

Unit Size: 1 labeling reaction of 1 mg antibody per kit

Kit Contents

Component	Size
Modified HRP (92437A)	1 vial (lyophilized solid)
HRP Reaction Buffer, 10X (99994-125uL)	125 uL
HRP Reaction Enhancer (99995)	1 vial (lyophilized solid)
Mix-n-Stain™ Quenching Buffer, 10X (99854-150uL)	150 uL

Storage and Handling

Store at -20°C. Kit is stable for at least 12 months from date of receipt when stored as recommended.

Product Description

The Mix-n-Stain™ Maxi HRP Antibody Labeling Kits allow you to rapidly label 1 mg of IgG antibody with horseradish peroxidase (HRP) in a little over 3 hours, with minimal hands-on time (see Protocol Overview). No purification is required after labeling. The HRP conjugate can be stored at 4°C for a few days after labeling; for longer term storage we recommend adding glycerol and BSA to stabilize the antibody; with stabilizers, the conjugate can be stored at -20°C for three months or longer.

This kit is not designed to be split for multiple labeling reactions. To label smaller amounts of antibody, use our Mix-n-Stain™ HRP Antibody Labeling Kits for labeling 10-20 ug, 25-50 ug, or 50-100 ug of antibody (see Related Products).

We also offer Mix-n-Stain™ Antibody Labeling Kits for conjugating antibodies to Biotium's bright and photostable CF® Dyes, fluorescent proteins (R-PE, APC, PerCP, and tandem dyes) or other enzymes (AP, and glucose oxidase). Our CF® Dye SE Protein Labeling Kits are designed for performing conjugation with post-labeling purification. Visit www.biotium.com for more information.

Before You Begin

Mix-n-Stain™ HRP Antibody Labeling Kits are optimized for labeling IgG antibodies. We do not recommend using them to label other proteins, because the ratio of HRP to protein may not be optimal. Mix-n-Stain™ labeling conditions may cause IgM antibodies to denature.

Antibodies should be at 1 mg/mL in PBS or similar buffer with no sodium azide, stabilizer proteins, glycerol, Tris, or amino acids such as glycine.

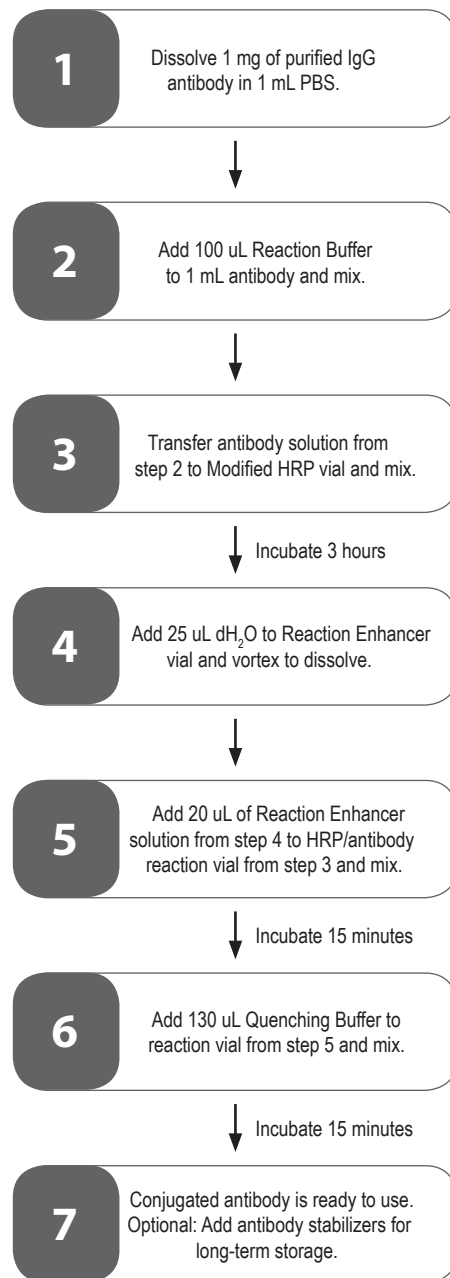
If your antibody concentration is higher than 1 mg/mL, add PBS to dilute it to 1 mg/mL. If your antibody concentration is lower than 1 mg/mL, concentrate the antibody to 1 mg/mL; a 10K MWCO ultrafiltration vial (see Related Products) can be used to concentrate your antibody. Ultrafiltration also can be used to remove incompatible small molecules such as azide, glycerol, Tris, or glycine (see Table 1).

Antibodies containing protein stabilizers such as BSA or gelatin, or crude antibodies in serum, culture supernatant, or ascites fluid should be purified before labeling using a standard Protein A/G or similar purification protocol (see Table 1; note: ultrafiltration will not remove protein stabilizers).

Table 1. Reaction Compatibility Guide

Component	Compatibility
Sodium Azide	Perform ultrafiltration (see Related Products)
Glycerol	Perform ultrafiltration (see Related Products)
Tris	Perform ultrafiltration (see Related Products)
Glycine	Perform ultrafiltration (see Related Products)
BSA or gelatin	Not compatible; purify antibody
Serum, cell culture supernatant, or ascites	Not compatible; purify antibody

Mix-n-Stain™ Maxi HRP Labeling Overview



Labeling Protocol

1. Prepare at least 1 mL of 1 mg/mL antibody solution in PBS or other compatible buffer (see Before You Begin for details). If your antibody is in lyophilized form, reconstitute in phosphate buffered saline (PBS).
2. Add 100 μ L of reaction buffer (99994) to 1 mL of antibody solution.
3. Add the solution from step 2 to the vial containing Modified HRP (92437A). Pipette the solution up and down to mix with the modified HRP.
4. Incubate the reaction at room temperature for 3 hours.
5. Add 25 μ L dH_2O to the vial containing HRP Reaction Enhancer (99995). Vortex to dissolve the enhancer.
6. Add 20 μ L of Reaction Enhancer solution from step 5 to the reaction from step 4 and vortex to mix.
7. Incubate at room temperature for 15 minutes.
8. Add 130 μ L Mix-n-Stain™ Quencher to the reaction and vortex to mix.
9. Incubate at room temperature for 15 minutes.
10. The antibody conjugate is now ready for use. 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.

Storage of Labeled Antibody

The HRP conjugated antibody can be stored at 4°C for up to 3 days. For longer-term storage, we recommend adding stabilizers to the antibody solution; for HRP conjugates we recommend adding glycerol to a final concentration of 50% and BSA to a final concentration of 15 mg/mL. Do not add sodium azide, which inhibits HRP activity. HRP conjugates with BSA and 50% glycerol can be stored at -20°C for at least 3 months; the glycerol will prevent the solution from freezing, so it is not necessary to aliquot the antibody for storage.

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
92300	Mix-n-Stain™ HRP Antibody Labeling Kit, 1x(10-20ug) labeling
92301	Mix-n-Stain™ HRP Antibody Labeling Kit, 1x(25-50ug) labeling
92302	Mix-n-Stain™ HRP Antibody Labeling Kit, 1x(50-100ug) labeling
29049	Streptavidin HRP
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 (Amplex® Red)
30015	DAB Substrate Kit
10050	ABTS

Please visit www.biotium.com to view our full selection of products including a wide selection of HRP-conjugated primary and secondary antibodies, CF® dye Mix-n-Stain™ antibody labeling kits, and CF® dye conjugates. Biotium also offers a full selection of dye-conjugated tyramides and CF® Dye Tyramide Amplification Kits.

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