Protocol

TD-P Revision 2.0

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Mix-n-Stain™ Biotin Antibody Labeling Kit

Procedure to Conjugate an Antibody to Biotin

Introduction

Mix-n-Stain™ antibody labeling kits contain everything you need to rapidly label an antibody with biotin. The labeling procedure comprises simple mixing of your antibody with the reaction buffer and the modified biotin provided, followed by a brief incubation. The Biotin is no longer reactive at the end of the labeling, so the conjugate is ready for staining without further purification. After labeling, biotin is covalently linked to the antibody with a degree of labeling of approximately 4-6 biotin molecules per antibody molecule.

Mix-n-Stain™ labeling can tolerate sodium azide, Tris, glycine and low levels of glycerol. A microcentrifuge ultrafiltration vial is provided in the kit to rapidly remove incompatible small molecule antibody stabilizers before labeling. The standard Mix-n-Stain™ labeling protocol can be performed in the presence of up to four-fold excess of BSA or gelatin to IgG (by µg amount). Simply choose the kit size that corresponds to the amount of IgG you wish to label. **A modified protocol is provided for labeling IgG in the presence of excess stabilizer protein or ascites fluid.** In this case, choose the kit size that corresponds to the total amount of protein (IgG + stabilizer, or total protein amount in ascites fluid) in the antibody sample you wish to label. The modified protocol also can be used to label amounts of IgG that fall below the lower range of the kit by adding stabilizer protein to the IgG to bring the total protein amount within the kit range. The modified protocol is not recommended for labeling antibodies in crude antiserum or hybridoma cell culture supernatant due to the low concentration of antibody relative to total protein in these formats.

When performing direct immunofluorescence with a fluorescently-labeled antibody, you may need to use a higher concentration of antibody to achieve similar staining intensity compared to indirect immunofluorescence detection using unlabeled primary plus labeled secondary antibody. Typically, indirect immunofluorescence staining results in about 3-fold signal amplification compared to direct immunofluorescence staining. Labeled secondary antibodies will still bind to primary antibodies labeled using Mix-n-Stain™ kits, therefore if multiple primary antibodies from the same species are to be used for multicolor immunofluorescence staining, a secondary antibody cannot be used to distinguish an unlabeled primary antibody from a Mix-n-Stain™ labeled primary antibody from the same species. Mix-n-Stain™ labeled antibodies can be used as a tertiary staining antibody after standard immunofluorescence staining with primary and secondary antibodies. Biotin Mix-n-Stain™ kits are great for use in secondary detection via streptavidin or with anti-biotin monoclonal antibodies.

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Materials

Table 1. Kit Components

Component	B-825-20 5-20 µg labeling	B-825-50 20-50 µg labeling	B-825-100 50-100 µg labeling
Modified Biotin	1 vial (Component A)	1 vial (Component A)	1 vial (Component A)
Mix-n-Stain™ reaction buffer, 10X	1 vial (15 µl)	1 vial (15 µl)	1 vial (30 µl)
Mix-n-Stain™ antibody storage buffer	1 vial (60 µl)	1 vial (150 µl)	1 vial (300 µl)
Ultrafiltration vial (MWCO=10K)	1 each	1 each	1 each

Storage/Handling

Store the kit at -20°C. The kit is stable for at least 3 months from date of receipt when stored as recommended.

Preliminary Notes

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. If your primary antibody is a commercial product, please contact the supplier to obtain the antibody concentration and formulation. Mix-n-Stain™ labeling cannot be used to label antibodies in crude serum or hybridoma supernatants. Use a Protein A purification procedure to purify IgG prior to labeling.

An antibody solution free of stabilizers produces the best labeling results, however, low levels of BSA, gelatin, Tris, glycerol in the antibody solution can be tolerated in the standard Mix-n-Stain™ labeling protocol. The labeling is not affected by sodium azide. For the standard Mix-n-Stain™ labeling protocol (Section B), select the kit size that corresponds to the total µg amount of IgG you wish to label.

The modified Mix-n-Stain™ labeling protocol (Section C) is based on the total amount of protein in the labeling reaction rather than the amount of IgG in the labeling reaction. The modified protocol should be used to label antibodies in the presence of excess stabilizer protein. Antibodies in ascites fluid can also be labeled using the modified protocol, however you must determine the concentration of total protein in the ascites fluid before labeling (estimation of protein concentration by measuring absorbance at 280 nm is sufficient). Select the kit size that

is appropriate for the total µg amount of protein in the antibody sample that you wish to label. The modified protocol also can be used to label antibody amounts that fall below the lower limit of the kit range by adding additional protein to the IgG to bring the total protein amount within the kit range.

Antibodies labeled in the presence of low levels of BSA and gelatin may show slightly higher background staining compared to antibody labeled without these stabilizers. If the antibody was labeled in the presence of BSA or gelatin, background staining can be greatly reduced by using blocking and wash solutions containing at least 1% BSA or gelatin, respectively.

To remove non-protein components such as Tris, glycine or glycerol, use the ultrafiltration vial provided in the kit to purify your antibody by following the steps in Section A.

The optimal antibody concentration for labeling is 0.5-1.0 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). If no antibody concentration is required, proceed to the standard antibody labeling protocol (Section B) or the modified antibody labeling protocol (Section C) as appropriate.

Table 2. Mix-n-Stain™ Antibody Compatibility and Labeling Protocol Selection Guide

Component	Compatibility
Sodium Azide	Compatible
Glycerol	≤10%: proceed to standard protocol (Section B) >10%: perform ultrafiltration (Section A)
Tris	≤20mM: proceed to standard protocol (Section B) >20mM: perform ultrafiltration (Section A)
Glycine	Perform ultrafiltration (Section A)
BSA or gelatin	≤4X IgG by µg amount: use standard protocol (Section B) > 4X IgG by µg amount: use modified protocol (Section C)
Ascites fluid	Use modified protocol (Section C)
Serum	Not compatible; purify IgG
Hybridoma cell culture supernatant	Not compatible; purify IgG

Method

A. Ultrafiltration Protocol

Note: Before you begin, use Table 2 (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 appropriate labeling protocol indicated in Table 2.

Note: The ultrafiltration column membrane has a molecular weight cut-off of 10,000 daltons. 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	Volume
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 0.5-1.0 mg/ml. Pipet the PBS carefully 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. If you are using the modified antibody labeling protocol, save the ultrafiltration vial to concentrate your antibody after labeling.

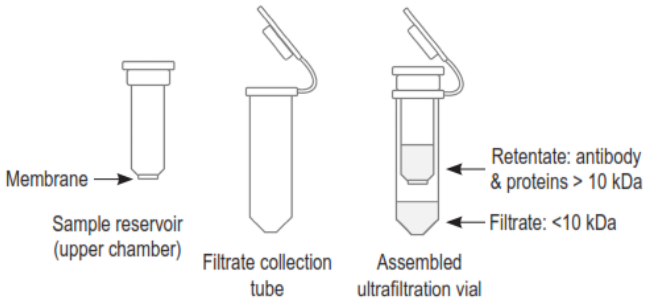

Figure 1: Ultrafiltration vial components

B. Standard Mix-n-Stain™ Labeling Protocol

Note: Before you begin, use Table 2 (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.

1. Use your antibody at a concentration of 0.5-1.0 mg/ml for optimal labeling. If the antibody is in a lyophilized form or is more concentrated, reconstitute or dilute the antibody in PBS. Transfer the antibody to be labeled to a clean tube. Make sure the µg amount of IgG in the labeling reaction falls within the range of the kit.

2. Warm up the Mix-n-Stain™ Reaction Buffer vial and the Mix-n-Stain™ Storage Buffer vial to room temperature before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.

3. Mix the 10X Mix-n-Stain™ Reaction Buffer with the antibody solution at a ratio of 1:10 so that the antibody solution contains a final concentration of 1X Reaction Buffer (for example, mix 9 µl of antibody with 1 µl of 10X reaction buffer). Mix the solutions by pipetting up and down a few times.

4. Transfer the entire solution from Step 3 to the vial containing the modified biotin. There is no need to measure the amount of the biotin in the vial. Vortex the vial for a few seconds.

5. Incubate the vial in the dark for 30 minutes at room temperature.

6. Dilute the labeled antibody solution with the provided Storage Buffer. Simply transfer the entire labeled antibody solution into the Storage Buffer. The antibody is now ready to use for staining. The concentration of the biotin-labeled antibody is approximately the amount of your starting antibody divided by the total volume.

   Note: Antibody Storage Buffer contains 2mM sodium azide.

7. The labeled antibody is stable for at least 6 months when stored 4°C, protected from light. Alternatively, the antibody can be stored in single use aliquots at -20°C for longer term storage.

   Note: If you prefer not to use the antibody dilution buffer, you can store the solution in single use aliquots at -20°C. Without repeated freeze-thaws, the labeled antibody solution should be stable for at least 6 months.

C. Modified Mix-n-Stain™ Labeling Protocol

Note: Before you begin, use Table 2 (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.

1. Use your antibody solution at a concentration of 0.5-1.0 mg/ml total protein (IgG plus stabilizer protein) for optimal labeling, using PBS to dilute the solution if necessary. Make sure the µg amount of total protein in the labeling reaction falls within the range of the kit. If you wish to label an amount of IgG that falls below the lower limit of the kit, add BSA to bring to the total protein concentration (IgG + BSA) within the range of the kit and proceed with labeling based on total protein concentration.

2. Warm up the Mix-n-Stain™ Reaction Buffer vial and the Mix-n-Stain™ Storage Buffer vial to room temperature before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.

3. Mix the 10X Mix-n-Stain™ Reaction Buffer with the antibody solution at a ratio of 1:10 so that the antibody solution contains a final concentration of 1X Reaction Buffer (for example, mix 9 µl of antibody with 1 µl of 10X reaction buffer). Mix the solutions by pipetting up and down a few times.

4. Transfer the entire solution from Step 3 to the vial containing the modified biotin. There is no need to measure the amount of the biotin in the vial. Vortex the vial for a few seconds.

5. Incubate the vial in the dark for 30 minutes at room temperature.

6. **Optional**: you can transfer the entire labeling reaction to the tube of antibody storage buffer provided. However, this may result in a highly dilute IgG solution, which may not be practical for subsequent use. To transfer the antibody to storage buffer without additional dilution, follow the steps below.

   Note: Antibody Storage Buffer contains 2mM sodium azide.

7. Transfer the labeling reaction to the membrane of the ultrafiltration vial provided (or saved after antibody clean-up, above. Centrifuge the vial at 14,000 x g until all of the liquid has filtered into the receiving vial as described in section A.

8. Resuspend the labeled antibody in antibody storage buffer at the desired final concentration of IgG. Carefully pipette the storage buffer up and down over the upper surface of the membrane to recover and resuspend the antibody.

   Note: Antibody Storage Buffer contains 2mM sodium azide.

9. Transfer the recovered antibody solution to a fresh microcentrifuge tube. The antibody is now ready to use for staining.

10. The labeled antibody is stable for at least 6 months when stored 4°C, protected from light. Alternatively, the antibody can be stored in single use aliquots at -20°C for longer term storage.

Table 4. Other Mix-n-Stain™ Antibody Labeling Kits

GoldBio Catalog #	Product Name
A-825	Mix-n-Stain™ AP Antibody Labeling Kit
F-825	Mix-n-Stain™ FITC Antibody Labeling Kit
G-825	Mix-n-Stain™ Glucose Oxidase (GOX) Antibody Labeling Kit
H-825	Mix-n-Stain™ HRP Antibody Labeling Kit