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Product Information

Mix-n-Stain™ Biotin Antibody Labeling Kits

Catalog Numbers **MXBIOS20, MXBIOS50, and MXBIOS100**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

This kit contains everything you need to rapidly label an antibody with biotin for secondary detection using an enzymatically or fluorescently labeled anti-biotin antibody or streptavidin. Select the labeling kit suitable for the amount of antibody to be labeled. The labeling procedure is simply mixing of the antibody with the optimally formulated biotin reagent in the reaction buffer provided, followed by a brief incubation. The resulting solution is ready for staining without further purification. The kit is suitable for labeling commercially available primary antibodies, either directly or after a simple antibody clean-up step. After labeling, biotin is covalently linked to the antibody with a degree of labeling of 4–6 biotin molecules per antibody molecule. Multiple antibodies labeled with biotin or CF™ dyes using different Mix-n-Stain™ kits can be used for multicolor fluorescence staining without transfer of biotin or dyes between antibodies.

Note: Labeled secondary antibodies will 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.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at –20 °C.

Procedure

Antibody Preparation

Check the compatibility of the antibody with the compatibility table (see Table 1). An antibody solution free of stabilizers produces better labeling results. However, low levels of BSA, gelatin, Tris, glycerol, or sodium azide in the antibody solution can be tolerated.

Table 1.
Mix-n-Stain Compatibility with Common Antibody Storage Components

Component	Compatibility
BSA or gelatin	<4 equivalents of antibody by weight
Glycerol	<10%
Glycine	not compatible
Serum	not compatible
Sodium Azide	compatible
Tris	<20 mM

If the stabilizers are above the compatibility threshold concentrations, purify the antibody.

Although low levels of BSA and gelatin can be tolerated, one may experience slightly higher background after staining if the antibody is labeled in the presence of these proteins. Background staining can be greatly reduced by using blocking and wash solutions containing at least 1% BSA or gelatin, respectively.

Protein stabilizers (BSA or gelatin) can be removed using an antibody clean-up kit.

To remove non-protein components (Tris, glycine, or glycerol) use the ultrafiltration vial provided in the kit (proceed to step 1a).

If no clean-up is required, proceed to step 2.

Note: The ultrafiltration column membrane has a molecular mass cut-off of 10,000 Da. 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. Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of antibody.

1a. Add an appropriate amount of antibody to the ultrafiltration vial, being careful not to touch the membrane. Spin the solution at maximal speed (14,000 rpm) in a microcentrifuge for a few minutes until the liquid is removed. Discard the liquid in the collection vessel.

1b. Add an equal volume of 1× PBS to the membrane. Spin the vial at maximal speed until the liquid is removed.

1c. Add an appropriate concentration of PBS to the membrane to obtain a final antibody concentration of 0.5–1 mg/mL. Carefully pipette the PBS up and down over the upper surface of the membrane to recover and resuspend the antibody. Transfer the antibody solution to a clean microcentrifuge tube.

2. For optimal labeling, use the antibody at a concentration of 0.5–1 mg/mL. If the antibody is lyophilized or is more concentrated, reconstitute or dilute the antibody in PBS. If your primary antibody is a commercial product with no quantity information, please contact the supplier. Resuspend the antibody in an appropriate volume of 1× PBS.

Antibody Labeling

1. 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.
2. Pipette an appropriate volume of antibody solution into a microcentrifuge tube.
3. Mix the 10× Mix-n-Stain Reaction Buffer with the antibody solution at a ratio of 1:9, so the antibody solution contains a final concentration of 1× Reaction Buffer (for example, mix 9 µL of antibody solution with 1 µL of 10× 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 biotin. There is no need to measure the amount of biotin in the vial. Vortex the vial for a few seconds.
5. Incubate the vial in the dark for 30 minutes. The solution is now ready to use. The concentration of the biotin-labeled antibody is approximately the amount of starting antibody divided by the total volume (i.e., ~100% labeling yield).
6. Dilute the labeled antibody solution with the provided Storage Buffer. Simply transfer the entire labeled antibody solution into the Storage Buffer and store at 2–8 °C. Recalculate the concentration of the antibody solution.
   Note: If you prefer not to use the antibody dilution buffer, you can aliquot the solution and store at –20 °C. Without repeated freeze-thaws the labeled antibody solution remains active for at least 6 months.

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