MM Biotinylation System

For Mouse on Mouse IHC Staining

Detection Kit

Control Number: 902-MMBK-081315

Catalog Number: MMBK H

Description: 25 ml

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

Summary & Explanation:
MM Biotinylation Kit is a biotinylated detection specially formulated to eliminate endogenous mouse IgG background staining when using a mouse primary antibody on mouse tissue. Even the most difficult tissues such as lymphatic, lung and kidney tissues are virtually background free.
When a mouse monoclonal antibody is desired for immunohistochemical detection on murine tissues, the biotinylated secondary antibody used for detection will bind to the primary antibody and will bind to endogenous mouse IgG in the tissue. Troublesome background staining may occur from bound mouse IgG.
The majority of primary antibodies on the market are not biotinylated. It is very expensive and time consuming to biotinylate an antibody. Usually, a minimum 2-5 mg of antibody is necessary for the procedure, and the total amount (mg/ml) of the antibody is reduced through the process.
To circumvent the problem, the MM Biotinylation kit provides all the reagents necessary to biotinylate a primary antibody. Prior to the application of primary antibody, 5 to 10 ul of antibody is mixed with a biotinylation reagent for 30 minutes. The biotinylated primary antibody complex can be immediately applied to the tissue specimen. The tissue is then incubated with streptavidin-peroxidase, and the color-reaction is developed by a diaminobenzidine /hydrogen peroxide chromogen-substrate (DAB).

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

25ml Kit:
1. Da Vinci Green (PD900H) 25ml
2. Background Sniper (BS966H) 25ml
3. Peroxidazed 1 (PX968H) 25ml
4. Biotinylation Reagent (MMBR610F) 4ml
5. Mopping Reagent (MMMR611F) 2x4ml
6. Streptavidin-HRP Label (HP604H)
7. DAB Chromogen (DB851D)
8. DAB Substrate Buffer (DS854H) 2x25ml
9. CAT Hematoxylin (CATHEH) 25ml
10. DAB Sparkle (DS830H) 25ml
11. Mixing Vial (VL103)

Materials and Reagents Needed But Not Provided:

Super PAP Pen
Microscope slides, positively charged
Desert Chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
Decloaking Chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*(TBS/PBS)
Pretreatment reagents*
Enzyme digestion*
Avidin-Biotin Blocking Kit*(Labeled Streptavidin Kits Only)
Primary antibody*
Negative control reagents*
Bluing reagent*
Mounting medium*

* Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Species Reactivity:

N/A

Storage and Stability:

Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Staining Protocol Recommendations:

Slide Preparation: (Paraffin-Embedded)
1. Cut tissues 4 microns thick. (Do not use adhesives in the water bath).
2. Place the tissue sections on a positive-charged slide.
3. Drain excess water off the slides
4. Dry at 37ºC to 40ºC overnight. Then dry slides for 15-30 minutes at 60ºC.

Preparation for the Biotinylation of a Primary Antibody:
1. Add the proper quantities of Diluent, Concentrated Primary Antibody, and Biotinylation Reagent in a test tube as calculated on the Reagent Worksheet. Mix for 15 to 30 minutes.
2. Then add the Mopping Reagent to the test tube as calculated on the Reagent Worksheet. Mix for 15 minutes.
   The primary antibody is biotinylated and ready-to-use. The antibody should be used within one to two days of preparation. If long-storage of the biotinylated primary antibody is desired, omit the Mopping Reagent and store at 4ºC. The biotinylated primary antibody without Mopping Reagent is stable for several months. Shelf life may vary, and ultimately, must be determined by the individual investigator.

Staining Procedure:
1. Deparaffinize tissue sections in 2 changes of xylene for 5 minutes each.
2. Hydrate slides in a graded series of alcohol (100%, 95% and 70 %) to water.
3. Apply Peroxidazed 1 for 5 minutes at room temperature (RT). Wash in tap water.
4. Rinse slides in deionized (DI) water.
5. Optional: If slides fixed in B-5, remove mercury pigment with hypo. Wash in tap water and then rinse in DI water.
6. Heat-Retrieval (Optional): Heat designated slide(s) in Biocare's Diva, Antigen Decloaker, Reveal, Nuclear Decloaker or Borg Decloaker (Biocare's Decloaking Chamber). Wash in tap water and rinse in DI water.
7. Make a square hydrophobic barrier around each tissue section with PAP Pen.
8. Add several drops of DI water onto the tissue section to keep from drying out.
9. Proteolytic Digestion (Optional): Digest designated tissues with Trypsin or Pepsin. Wash in tap water and rinse in DI water.
10. Rinse and wash in two changes PBS/TBS wash buffer for 5 minutes.
11. Optional: Apply 4 drops (approximately 150 µl) of Biocare's Avidin Block for 15 minutes at RT. Rinse off slides with PBS/TBS wash buffer.
12. Optional: Apply 4 drops (approximately 150 µl) of Biocare's Biotin Block for 15 minutes at RT. Rinse off slides with PBS/TBS wash buffer.
13. Apply 4 drops (approximately 150 µl) of Biocare's Background Sniper for 10 minutes at RT.
14. Rinse off slides with PBS/TBS wash buffer.
15. Apply 4 drops of prepared Biotinylated Primary Antibody for 60 minutes at RT. Apply 4 drops of prepared Biotinylated Negative Control Serum to the negative control tissue.
16. Rinse and wash in PBS/TBS wash buffer for 5 minutes with agitation.
17. Apply 4 drops of the Streptavidin Horseradish-Peroxidase (HRP). Incubate for 15 minutes at RT.
18. Rinse and wash in PBS/TBS wash buffer for 5 minutes with agitation.
19. Apply 4 drops of the DAB Chromogen Substrate. Develop 5 minutes at RT. Wash in DI water.
20. DAB Sparkle (Optional): Apply 4 drops for 1 minute and rinse in DI water.
21. Apply 4 drops of CAT Hematoxylin for 1 to 2 minutes.
22. Wash in DI or tap water.
23. Blue nuclei in Tacha's Bluing Solution for 30 seconds to 1 minute.
24. Wash in DI or tap water.
25. Dehydrate in 3 changes of 100% alcohol.
26. Clear in 3 changes of clearing agent.
27. Mount and coverslip.

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Technical Notes:

Tissue controls

Tissue controls can either be positive or negative. Controls should be prepared identically to the unknown (test sample).

Positive Control Slides

Positive control tissue should be prepared from tissue known to contain the appropriate antigen and processed in the same manner as the test tissue. A positive control is used to determine if the proper sequence of reagents was applied correctly. It is recommended, if possible, to place the positive control tissue on the same slide as the test sample.

Negative Control Slides

Substitute the primary antibody with a negative control reagent. This will determine any non-specific background staining that may occur due to cross-reactivity of reagents or endogenous peroxidase or artifacts. A negative control tissue should be from the same tissue source and/or tissue block as the test sample. Substitute the biotinylated primary antibody with the negative control reagent. The negative control reagent should be same isotype and IgG concentration as the primary antibody (not total protein concentration). Prepare and calculate the negative control reagent the same way as the biotinylated primary antibody (see Reagent Worksheet). An alternative way to prepare a negative control reagent is to omit the primary antibody from the Biotinylation Reagent.

Staining Protocol Notes:

Tissue Fixation and Processing

Proper fixation of tissue samples is extremely important. An appropriate fixative is required to obtain optimum performance and reliable interpretations. The following are commonly used fixatives: 10% neutral buffered formalin, 4% paraformaldehyde, zinc formalin, Zamboni's and alcohol-based fixatives.

Cut tissue samples at 3 to 5 mm thickness. Carefully select the appropriate fixative and fix tissues for 4 to 8 hours. A panel of the above fixatives may be used to determine optimum morphology and antibody-antigen interaction. When using crosslinking fixatives such as formalin or paraformaldehyde, do not over-fix tissue samples. This may cause excessive crosslinking of target antigens and prevent antibody access.

After fixation, process tissues through a graded series of alcohol (70%, 80%, 95% and 100%). Clear in xylene or a xylene substitute and infiltrate with paraffin.

When processing rodent tissues, over dehydration and clearing may cause excessive shrinkage and make tissues brittle and hard to cut. Therefore, a shorter processing time is recommended. Total processing time should be no longer than 3 to 4 hours after fixation.

Frozen Tissues

Frozen sections should be cut from snap frozen tissue blocks (freeze in isopentane at approximately -140ºC. to -150ºC). Cut frozen sections at 3-5 microns and dry for 1 hour at room temperature. Fix frozen section 100% acetone or methanol at 4ºC for 10 minutes. There are certain procedures that may require a crosslinking fixative such as 4% paraformaldehyde. Fix frozen sections in cold (4ºC) 4% paraformaldehyde in PBS for 10 minutes. Wash sections in 2 changes of cold PBS. Post-fix in cold methanol for 4 minutes and then place directly into cold acetone for two minutes. Rinse in two changes of PBS.

Limitations:

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

Precautions:

1. This product is not classified as hazardous. The preservative used in this reagent is Proclin 950 and the concentration is less than 0.25%. Overexposure to Proclin 950 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 950 in this product does not meet the OSHA criteria for a hazardous substance.
2. Wear disposable gloves when handling reagents.
3. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions.
4. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
5. Microbial contamination of reagents may result in an increase in nonspecific staining.
6. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
7. The SDS is available upon request and is located at http://biocare.net.
8. Consult OSHA, federal, state or local regulations for disposal of any toxic substances. Proclin™ is a trademark of Rohm and Haas Company, or of its subsidiaries or affiliates.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

Troubleshooting Guide:

No Staining

1. Critical reagent (such as primary antibody) omitted.
2. Staining steps performed incorrectly or in the wrong order.
3. Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, the wrong order or the wrong pretreatment.
4. Insufficient amount of antigen.
5. Secondary antibody at too low of a concentration.
6. Primary antibody incubation period too short.
7. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining

1. Tissue is either over-fixed or under-fixed.
2. Incubation time of primary antibody too short
3. Low expression of antigen.
4. Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment.
5. Over-development of substrate.
6. Excessive rinsing during wash steps.
7. Omission of critical reagent.
8. Incorrect procedure in reagent preparation.
9. Improper procedure in test steps.

Non-specific or High Background Staining

1. Tissue is either over-fixed or under-fixed.
2. Endogenous alkaline phosphatase or peroxidase (not effectively blocked).
3. Endogenous biotin in specimen (not blocked with avidin biotin blocking agent).
4. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
5. Tissue may need a longer or a more specific protein block.
6. Substrate is overly-developed.
7. Tissue was inadequately rinsed.
8. Deparaffinization incomplete.
9. Tissue damaged or necrotic.

Tissues Falling Off

1. Slides were not positively charged.
2. A slide adhesive was used in the waterbath.
3. Tissue was not dried properly.
4. Tissue contained too much fat.

Specific Staining Too Dark

1. Concentrated antibody not diluted out properly (being used at too high of a concentration).
2. Incubation of primary antibody, link or label too long.

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Reagent Preparation:

To biotinylate a primary antibody the following parameters and calculations must be determined (see Reagent Worksheet):

1. Working Volume

Multiply the number of slides required times the working volume per slide:

Number of slides x µl/slide = Working Volume:

2. Volume of Concentrated Primary Antibody

The initial titer of the primary antibody may include a range of dilutions. The sensitivity threshold of this kit has been determined to be approximately 0.5-1.0 ug /ml for most antibodies. Example: If the IgG concentration of the concentrated primary antibody is 100ug/ml, the suggested titration range would be approximately 1:50 to 200. At 1:100 the IgG concentration would be 1ug/ ml. Once the final titration has been calculated, the volume of concentrated primary antibody can be determined as the Dilution Factor:

Volume of Working Solution x Dilution Factor = Volume of Concentrated Primary Antibody

3. Volume of Biotinylation Reagent

To properly determine the amount of the Biotinylation Reagent required, the IgG concentration (not total protein concentration) of the primary antibody must be known. It is usually expressed in ug/ml or mg/ml. Divide the IgG concentration by 50, and then multiply the Volume of Concentrated Primary Antibody used:

IgG Concentration ÷ 50 x Volume of Concentrated Primary Antibody = Volume of Biotinylation Reagent

4. Volume of Mopping Reagent

To determine the Volume of Mopping Reagent required, divide the Working Volume (see number 1) by 25.

Working Volume ÷25= Volume of Mopping Reagent

5. Volume of Reagents

To determine the Volume of Reagents, add the total volume or reagents used:

Volume of Concentrated Primary Antibody + Volume of the Biotinylated Reagent + Volume of Mopping Reagent = Volume of Reagents.

6. Volume of Diluent

To determine the Volume of Diluent, subtract the Volume of Reagents from the Working Volume:

Working Volume-Reagent Total= Antibody Diluent

Sample Reagent Worksheet

To biotinylate a primary antibody, an accurate calculation of several factors is required. Below is an example of a Reagent Worksheet.

1. Working Volume

5 Number of Slides x **200** µl Volume/Slide = **1000** µl Working Volume

2. Volume of Concentrated Primary Antibody

1000 µl Working Volume ÷ **50** (1:50) Dilution Factor = **20 µl** Primary Antibody

3. Volume of Biotinylation Reagent

50 µg/ml IgG Concentration of Primary Antibody ÷ **(50)** x **20** Volume of Concentrated Primary Antibody = **20** µl Biotinylation Reagent

4. Volume of Mopping Reagent

1000 µl Working Volume ÷ **(25)** = **40** µl Mopping Reagent

5. Volume of Reagents

20 µl Volume of Concentrated Primary Antibody + **20 µl** Volume of Biotinylation Reagent + **40 µl** Volume of Mopping Reagent = **80 µl** Total Volume

6. Volume of Diluent

1000 µl Working Volume − **80 µl** Reagent Volume = **920** µl Total Volume of Diluent

7. Final Volume of Reagents and Diluent

80 µl Volume of Reagents + **920 µl** Volume of Diluent = **1000 µl** Total Volume of Reagents and Diluent

Reagent Worksheet

Primary Antibody: _____ Source:

Lot Number: _____ IgG Concentration:

Dilution Factor: _____(_) Final Concentration:_____µg/ml

Performed By: _____ Date: _____ Reviewed By:

1. Working Volume

_____ Number of Slides x _____µl Volume/Slide = _____ µl Working Volume

2. Volume of Concentrated Primary Antibody

_____ µl Working Volume ÷ _____ Dilution Factor = _____µl Primary Antibody

3. Volume of Biotinylation Reagent

_____ µg/ml IgG Concentration of Primary Antibody ÷ **(50)** x _____ Volume of Concentrated Primary Antibody = _____µl Biotinylation Reagent

4. Volume of Mopping Reagent

_____ µl Working Volume ÷ **(25)** = _____ µl Mopping Reagent

5. Volume of Reagents

_____ **µl** Volume of Concentrated Primary Antibody + _____ **µl** Volume of Biotinylation Reagent + _____ **µl** Volume of Mopping Reagent = _____**µl** Total Volume

6. Volume of Diluent

_____ **µl** Working Volume − _____ **µl** Reagent Volume = _____ µl Total Volume of Diluent

7. Final Volume of Reagents and Diluent

_____**µl** Volume of Reagents + _____ **µl** Volume of Diluent = _____ **µl** Total Volume of Reagents and Diluent

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