



MM Biotinylation System

For Mouse on Mouse IHC Staining

Detection Kit

Control Number: 902-MMBK-102710

ISO
9001:2000
CERTIFIED

Catalog Number: MMBK G, H

Description: 6.0, 25 ml

Intended Use:

For Research Use Only

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 biotinylating 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). The kit contains Da Vinci Green, Background Sniper, Peroxidized 1, Biotinylation Reagent, Mopping Reagent, Streptavidin-HRP Label, DAB Chromogen, DAB Substrate Buffer, CAT Hematoxylin, DAB Sparkle, and Mixing Vial.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

6ml Kit:

1. Da Vinci Green (PD900S) 6ml
2. Background Sniper (BS966G) 6ml
3. Peroxidized 1 (PX968G) 6ml
4. Biotinylation Reagent (MMBR610C) 1ml
5. Mopping Reagent (MMMR611D) 2ml
6. Streptavidin-HRP Label (HP604G) 6ml
7. DAB Chromogen (DB851C) 1ml
8. DAB Substrate Buffer (DS854G) 3x6ml
9. CAT Hematoxylin (CATHEG) 6ml
10. DAB Sparkle (DS830G)
11. Mixing Vial (VL103)

25ml Kit:

1. Da Vinci Green (PD900H) 25ml
2. Background Sniper (BS966H) 25ml
3. Peroxidized 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:

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*

Materials and Reagents Needed But Not Provided cont'd:

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 2°C to 8°C. 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 2°C to 8°C.

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 37°C to 40°C overnight. Then dry slides for 15-30 minutes at 60°C.

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 4°C. 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 Peroxidized 1 for 5 minutes at room temperature (RT). Wash in tap water.
4. Rinse slides in deionized water (D. I.).
5. Optional: If slides fixed in B-5, remove mercury pigment with hypo. Wash in tap water and then rinse in D. I. 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 D. I. water.
7. Make a square hydrophobic barrier around each tissue section with PAP Pen.
8. Add several drops of D. I. 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 D. I. water.
10. Rinse and wash in two changes PBS/TBS wash buffer for 5 minutes.
11. Apply 4 drops (approximately 150 µl) of Biocare's Background Sniper for 5 minutes at RT.
12. Rinse off slides with PBS/TBS wash buffer.
13. 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.
14. Rinse and wash in PBS/TBS wash buffer for 5 minutes with agitation.
15. Apply 4 drops of the Streptavidin Horseradish-Peroxidase (HRP). Incubate for 15 minutes at RT.
16. Rinse and wash in PBS/TBS wash buffer for 5 minutes with agitation.
17. Apply 4 drops of the DAB Chromogen Substrate. Develop 5 minutes at RT. Wash in D. I. water.
18. DAB Sparkle (Optional): Apply 4 drops for 1 minute and rinse in DI water.
19. Apply 4 drops of CAT Hematoxylin for 1 to 2 minutes.
20. Wash in DI or tap water.
21. Blue nuclei in Tacha's Bluing Solution for 30 seconds to 1 minute.
22. Wash in DI or tap water.
23. Dehydrate in 3 changes of 100% alcohol.
24. Clear in 3 changes of clearing agent.
25. Mount and coverslip.





MM Biotinylation System
For Mouse on Mouse IHC Staining
Detection Kit

ISO
9001:2000
CERTIFIED

Control Number: 902-MMBK-102710

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.

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 -140°C. to -150°C). Cut frozen sections at 3-5 microns and dry for 1 hour at room temperature. Fix frozen section 100% acetone or methanol at 4°C for 10 minutes. There are certain procedures that may require a crosslinking fixative such as 4 % paraformaldehyde. Fix frozen sections in cold (4°C) 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.

Performance Characteristics:

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:

Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on tissue controls.

Precautions:

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. Wear disposable gloves when handling reagents. 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. 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. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request. 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.

Troubleshooting:

Follow the reagent specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

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. Variable fixation time.
2. Endogenous alkaline phosphatase (not blocked with levamisole).
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.

Limitations & Warranty:

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.





MM Biotinylation System
For Mouse on Mouse IHC Staining
Detection Kit

ISO
9001:2000
CERTIFIED

Control Number: 902-MMBK-102710

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:

$$\text{Number of slides} \times \mu\text{l/slide} = \text{Working Volume}$$

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:

$$\text{Volume of Working Solution} \times \text{Dilution Factor} = \text{Volume of Concentrated Primary Antibody}$$

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 = 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.

$$\text{Working Volume} \div 25 = \text{Volume of Mopping Reagent}$$

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:

$$\text{Working Volume} - \text{Reagent Total} = \text{Antibody Diluent}$$

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

$$\frac{5}{\text{Number of Slides}} \times \frac{200}{\text{Volume/Slide}} \mu\text{l} = \frac{1000}{\text{Working Volume}} \mu\text{l}$$

2. Volume of Concentrated Primary Antibody

$$\frac{1000}{\text{Working Volume}} \mu\text{l} \div \frac{50 (1:50)}{\text{Dilution Factor}} = \frac{20}{\text{Primary Antibody}} \mu\text{l}$$

3. Volume of Biotinylation Reagent

$$\frac{50}{\text{IgG Concentration of Primary Antibody}} \mu\text{g/ml} \div (50) \times \frac{20}{\text{Volume of Concentrated Primary Antibody}} = \frac{20}{\text{Biotinylation Reagent}} \mu\text{l}$$

4. Volume of Mopping Reagent

$$\frac{1000}{\text{Working Volume}} \mu\text{l} \div (25) = \frac{40}{\text{Mopping Reagent}} \mu\text{l}$$

5. Volume of Reagents

$$\frac{20}{\text{Volume of Concentrated Primary Antibody}} \mu\text{l} + \frac{20}{\text{Volume of Biotinylation Reagent}} \mu\text{l} + \frac{40}{\text{Volume of Mopping Reagent}} \mu\text{l} = \frac{80}{\text{Total Volume}} \mu\text{l}$$

6. Volume of Diluent

$$\frac{1000}{\text{Working Volume}} \mu\text{l} - \frac{80}{\text{Reagent Volume}} \mu\text{l} = \frac{920}{\text{Total Volume of Diluent}} \mu\text{l}$$

7. Final Volume of Reagents and Diluent

$$\frac{80}{\text{Volume of Reagents}} \mu\text{l} + \frac{920}{\text{Volume of Diluent}} \mu\text{l} = \frac{1000}{\text{Total Volume of Reagents and Diluent}} \mu\text{l}$$

Reagent Worksheet

Primary Antibody: _____ Source: _____

Lot Number: _____ IgG Concentration: _____

Dilution Factor: _____ () Final Concentration: _____ ug/ml

Performed By: _____ Date: _____ Reviewed By: _____

1. Working Volume

$$\frac{\text{Number of Slides}}{\text{Number of Slides}} \times \frac{\text{Volume/Slide}}{\text{Volume/Slide}} \mu\text{l} = \frac{\text{Working Volume}}{\text{Working Volume}} \mu\text{l}$$

2. Volume of Concentrated Primary Antibody

$$\frac{\text{Working Volume}}{\text{Working Volume}} \mu\text{l} \div \frac{\text{Dilution Factor}}{\text{Dilution Factor}} = \frac{\text{Primary Antibody}}{\text{Primary Antibody}} \mu\text{l}$$

3. Volume of Biotinylation Reagent

$$\frac{\text{IgG Concentration of Primary Antibody}}{\text{IgG Concentration of Primary Antibody}} \mu\text{g/ml} \div () \times \frac{\text{Volume of Concentrated Primary Antibody}}{\text{Volume of Concentrated Primary Antibody}} = \frac{\text{Biotinylation Reagent}}{\text{Biotinylation Reagent}} \mu\text{l}$$

4. Volume of Mopping Reagent

$$\frac{\text{Working Volume}}{\text{Working Volume}} \mu\text{l} \div () = \frac{\text{Mopping Reagent}}{\text{Mopping Reagent}} \mu\text{l}$$

5. Volume of Reagents

$$\frac{\text{Volume of Concentrated Primary Antibody}}{\text{Volume of Concentrated Primary Antibody}} \mu\text{l} + \frac{\text{Volume of Biotinylation Reagent}}{\text{Volume of Biotinylation Reagent}} \mu\text{l} + \frac{\text{Volume of Mopping Reagent}}{\text{Volume of Mopping Reagent}} \mu\text{l} = \frac{\text{Total Volume}}{\text{Total Volume}} \mu\text{l}$$

6. Volume of Diluent

$$\frac{\text{Working Volume}}{\text{Working Volume}} \mu\text{l} - \frac{\text{Reagent Volume}}{\text{Reagent Volume}} \mu\text{l} = \frac{\text{Total Volume of Diluent}}{\text{Total Volume of Diluent}} \mu\text{l}$$

7. Final Volume of Reagents and Diluent

$$\frac{\text{Volume of Reagents}}{\text{Volume of Reagents}} \mu\text{l} + \frac{\text{Volume of Diluent}}{\text{Volume of Diluent}} \mu\text{l} = \frac{\text{Total Volume of Reagents and Diluent}}{\text{Total Volume of Reagents and Diluent}} \mu\text{l}$$

