



Vector® M.O.M.™ Immunodetection Kit

PEROXIDASE

Catalog No. PK-2200

Introduction

The Vector® M.O.M.™ Immunodetection Kit is designed specifically to localize mouse primary antibodies on mouse tissues. A major problem investigators have faced in attempts to use immunohistochemical techniques with mouse primary antibodies on mouse tissues is the inability of the anti-mouse secondary antibody to distinguish between the mouse primary antibody and any endogenous mouse immunoglobulins in the tissue. As a consequence there is high background staining which obscures the specific staining.

This background problem can be essentially eliminated by using the Vector® M.O.M.™ Immunodetection Kit which utilizes a novel blocking agent and special detection methodology to significantly reduce this undesired background staining. The Vector® M.O.M.™ Kit can be used with normal and genetically engineered mouse models, including transgenic, xenograft, knock out and other mutant strains.

COMPONENTS

The Vector® M.O.M.™ Immunodetection Kit contains:

- 6 ml of M.O.M.™ Protein Concentrate
- 1 ml Mouse IgG Blocking Reagent
- 0.1 ml M.O.M.™ Biotinylated Anti-Mouse IgG Reagent
- VECTASTAIN® *Elite* ABC Reagent A (1 ml) and Reagent B (1 ml)

The Vector® M.O.M.™ Immunodetection Kit contains enough stock reagents to produce about 25 ml of working solution which is generally sufficient to stain approximately 250 tissue sections.

Storage: The Vector® M.O.M.™ Kit should be stored at 2-8 °C. We recommend that the reagents be kept in the box in which they were supplied. If reagents are removed from the box please note on them the date shown on the box so that specific lots of reagents can be traced.

PREPARATION OF VECTOR® M.O.M.™ WORKING SOLUTIONS

- M.O.M.™ Mouse IgG Blocking Reagent: add 2 drops of stock solution to 2.5 ml of PBS or TBS. †
- M.O.M.™ Diluent: add 600 µl of Protein Concentrate stock solution to 7.5 ml of PBS or TBS. ††
- M.O.M.™ Biotinylated Anti-Mouse IgG Reagent: add 10 µl of stock solution to 2.5 ml of M.O.M.™ diluent prepared above.
- VECTASTAIN® *Elite* ABC Reagent: add 2 drops of Reagent A to 2.5 mls PBS or TBS. Mix. Then add 2 drops of Reagent B and mix. Allow *Elite* ABC Reagent to stand for 30 minutes prior to use.

◇ One drop is approximately 45 µl
† PBS: 10mM sodium phosphate, 0.15M NaCl, pH 7.4-7.8
TBS: 50mM TRIS, 0.15M NaCl, pH 7.5-7.8
†† Note: 7.5 ml of M.O.M.™ diluent provides sufficient reagent for use in steps 9, 10, and 12.

M.O.M.™ KIT STAINING PROCEDURE

1. Deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.
2. Rinse for 5 minutes in tap water.
3. Perform appropriate antigen unmasking, if required. For example, use Vector® Antigen Unmasking Solution, Cat. No. H-3300 (citrate-based) or Cat. No. H-3301 (high pH).
4. Block endogenous enzyme activity, if necessary, by incubating sections with BLOXALL™ Blocking Solution (Cat. No. SP-6000) for 10 minutes.* For alternative blocking protocols see Note 3.
5. Wash section 2 x 2 minutes in PBS or TBS.
6. Perform Avidin/Biotin blocking if required*, using Vector® Avidin/Biotin Blocking Kit (Cat. No. SP-2001).
7. Incubate sections for 1 hour in working solution of M.O.M.™ Mouse IgG Blocking Reagent prepared as described.
8. Wash sections 2 x 2 minutes in PBS or TBS**.
9. Incubate tissue sections for 5 minutes in working solution of M.O.M.™ diluent prepared as described**.
10. Tip excess of M.O.M.™ diluent off sections. Dilute primary antibody in M.O.M.™ diluent to the appropriate concentration. Incubate section in diluted primary antibody for 30 minutes**.
11. Wash sections for 2 x 2 minutes in PBS or TBS**.
12. Apply working solution of M.O.M.™ Biotinylated Anti-Mouse IgG Reagent prepared as described. Incubate sections for 10 minutes**.
13. Wash sections for 2 x 2 minutes in PBS or TBS.
14. Apply VECTASTAIN® ABC Reagent prepared as described. Incubate sections for 5 minutes.
15. Wash sections for 2 x 5 minutes in PBS or TBS.

16. Prepare and apply peroxidase substrate solution according to substrate kit instructions. For substrate choices see reverse.
- * When appropriate control sections have shown that endogenous peroxidase or endogenous avidin/biotin activity is not present, step 4 and/or step 6 may be omitted.
- ** It is recommended that the exact times described in steps 8-12 be used in the staining protocol. Longer incubation may result in an increase in background staining.

CUSTOMIZATION OF M.O.M.™ KIT PROTOCOL

Non-specific staining, at least in part, can be due to factors other than endogenous mouse IgG such as endogenous enzyme activity or non-specific protein interactions. Appropriate deletion controls should be done to determine the factors contributing to background staining. These controls are described in more detail in the general Troubleshooting Guide from Vector Laboratories, available on our website: www.vectorlabs.com.

The amount of endogenous mouse IgG will vary with tissue type, fixation method, fixative, and a variety of other factors. For the majority of mouse tissues, the dilution and incubation times recommended for the Vector® M.O.M.™ Kits and reagents are very effective in reducing the background caused by endogenous mouse IgG while maintaining high staining sensitivity.

The high sensitivity of Vector® M.O.M.™ detection reagents may require customizing the dilution of the Vector® M.O.M.™ Biotinylated Anti-Mouse IgG Reagent for tissues containing especially high levels of endogenous mouse IgG.

The concentration and/or the incubation time of the Vector® M.O.M.™ Mouse IgG Blocking Reagent may also be modified to optimize results.

For details see Vector® Troubleshooting Guide: Mouse Antibodies on Mouse Tissue, available on our website: <http://www.vectorlabs.com/Protocols/Supprotocols/MOMtsg.pdf>.

NOTES:

1.

The Vector® M.O.M.™ Biotinylated Anti-Mouse IgG in this kit recognizes both heavy and light chains of mouse IgG. Consequently, this kit can also be used to localize mouse IgM primary antibodies.
2.

Sections which are thicker than normal may require longer incubation times for optimal staining. Appropriate control slides should be run in parallel if incubation times are altered.
3.

Alternative protocols for blocking endogenous peroxidase:

For paraffin sections - incubate sections with 3% hydrogen peroxide in tap water for 5 minutes.

For frozen sections - incubate sections with 0.3% hydrogen peroxide in 0.3% Normal Horse Serum in PBS or TBS for 5 minutes.
4.

If staining is absent in formalin-fixed tissue, unmasking of antigens may be required. Antigen Unmasking Solutions, Citrate-based (Cat. No. H-3300) or High pH (Cat. No. H-3301) are recommended.
5.

Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used to prepare the peroxidase substrate or added to the VECTASTAIN® *Elite* ABC Reagent. This may result in reduced sensitivity.
6.

Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining. It is recommended that solutions be prepared with glass distilled water. Deionized water (even with low conductivities) may contain enzyme inhibitors of peroxidase and can reduce sensitivity.
7.

To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (Cat. No. SP-1800), a non-protein tissue section adhesive.

PEROXIDASE SUBSTRATES

A variety of chromogens can be used to localize peroxidase in tissue sections. All Vector Laboratories’ substrates are supplied in convenient, easy to use drop-per bottles. Vector Labs offers conventional as well as proprietary substrates producing the colors listed.

ImmPACT™ DAB <i>EqV</i> (Brown)	SK-4103	400 ml
ImmPACT™ DAB (Brown)	SK-4105	120 ml
ImmPACT™ AEC (Red)	SK-4205	120 ml
ImmPACT™ AMEC Red (Red)	SK-4285	120 ml
ImmPACT™ VIP (Purple)	SK-4605	120 ml
ImmPACT™ SG (Blue-Gray)	SK-4705	120 ml
ImmPACT™ NovaRED™ (Red)	SK-4805	120 ml
DAB (Brown or Gray-Black)	SK-4100	1 kit
AEC (Red)	SK-4200	1 kit
Vector® VIP (Purple)	SK-4600	1 kit
Vector® SG (Blue-Gray)	SK-4700	1 kit
Vector® NovaRED™ (Red)	SK-4800	1 kit
TMB (Blue)	SK-4400	1 kit

* AEC, ImmPACT™ AEC and ImmPACT™ AMEC Red are soluble in alcohol and clearing agents and must be mounted in aqueous mounting media. All other substrates are not soluble in alcohol or clearing agents. They may be dehydrated, cleared, and permanently mounted.

These substrates can be used as single labels or to introduce multiple colors in a tissue section.

Note: A chart of the Relative Sensitivity of Substrates in Immunohistochemistry and further description of substrate properties is available on our website: <http://www.vectorlabs.com/catalog.aspx?catID=163>

ADDITIONAL VECTOR® M.O.M.™ REAGENTS AND KITS

Vector® M.O.M.™ Mouse IgG Blocking Reagent	MKB-2213	1 ml
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This product contains the same reagent as that included in the M.O.M.™ kits.

Vector® M.O.M.™ Biotinylated Anti-Mouse IgG Reagent	MKB-2225	0.1 ml
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This product contains the same reagent as that included in the M.O.M.™ kits.

Vector® M.O.M. ImmPRESS™ Reagent	MPX-2402	15 ml
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This product contains the same reagent as that included in the M.O.M.™ ImmPRESS™ Peroxidase Kit.

Vector® M.O.M.™ Basic Kit	BMK-2202	1 kit
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This kit contains M.O.M.™ Mouse IgG Blocking Reagent, M.O.M.™ Biotinylated Anti-Mouse IgG Reagent, and the M.O.M.™ Protein Concentrate.

Vector® M.O.M.™ Fluorescein Kit FMK-2201	1 kit
This kit contains M.O.M.™ Mouse IgG Blocking Reagent, M.O.M.™ Biotinylated Anti-Mouse IgG Reagent, the M.O.M.™ Protein Concentrate and Fluorescein Avidin DCS.	

Vector® M.O.M.™ ImmPRESS™ Peroxidase Kit	MP-2400	1 kit
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This kit contains M.O.M.™ Mouse IgG Blocking Reagent, R.T.U. M.O.M.™ Serum Block, R.T.U. M.O.M.™ ImmPRESS™ Anti-Mouse IgG Reagent.

Blocking Serum		
Normal Horse	S-2000	20 ml
2.5 % Normal Horse	S-2012	50 ml

Sera are obtained from healthy adult animals, heat treated at 56 °C for 2 hours, incubated at 4 °C to precipitate cryoglobulins, ultracentrifuged and ultrafiltered through a 0.45µ filter.

BLOXALL™ Blocking Solution	SP-6000	100 ml
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BLOXALL™ inactivates endogenous peroxidase, pseudoperoxidase, and alkaline phosphatase in formalin-fixed, paraffin-embedded tissue sections, frozen tissue sections, and cell preparations. BLOXALL™ Blocking Solution is provided ready-to-use in a convenient dropper bottle.

Avidin/Biotin Blocking Kit	SP-2001	1 kit
Streptavidin/Biotin Blocking Kit	SP-2002	1 kit

These blocking kits consist of 18 ml of Avidin D or Streptavidin and 18 ml biotin in convenient dropper bottles. These kits are designed for use in those cases when streptavidin, avidin, or biotinylated reagents bind non-specifically to tissues or proteins.

VECTABOND™ Reagent	SP-1800	7 ml
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VECTABOND™ Reagent is designed to significantly increase adherence of both frozen and paraffin embedded tissue sections to glass slides during standard immunohistochemical procedures, or under harsh conditions such as required for high temperature antigen unmasking techniques. This product chemically modifies the glass to form a highly adherent surface. VECTABOND™ Reagent is provided as a 50x concentrated stock sufficient for treating at least 500 slides.

ImmEdge™ Pen	H-4000	2-pen set
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The ImmEdge™ Pen is designed to provide a pale blue, hydrophobic heat-stable barrier that keeps reagents localized to tissue sections.

ImmPrint™ Histology Pen	H-6100	5-pen set
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This black permanent marking pen is resistant to most organic solvents encountered in histological applications and is designed to write on glass slides, tissue cassettes, and most hard surfaces.

Antigen Unmasking Solution		
Citrate-based	H-3300	250 ml
High pH	H-3301	250 ml

These formulas are highly effective at revealing antigens in formalin-fixed, paraffin-embedded tissue sections using a high temperature treatment procedure. Antigen Unmasking Solutions are supplied as an approximately 100x concentrated stock sufficient to prepare 25 liters of working solution.

VectaMount™ Mounting Medium H-5000	60 ml
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This toluene-free permanent mounting medium contains no hazardous chemicals, is odorless, dries clear with an ideal refractive index, and shows no evidence of altering the color or intensity of any commonly used enzyme substrate.

VectaMount™ AQ Mounting Medium H-5501	60 ml
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This aqueous hard-setting mounting medium is designed for use with enzyme substrates, such as AEC, ImmPACT™ AEC or ImmPACT™ AMEC Red, whose reaction products are soluble in alcohol or other organic solvents.

COUNTERSTAINS

Vector® Hematoxylin	H-3401	500 ml
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Hematoxylin stains nuclei blue-violet with crisp nuclear detail. Our hematoxylin is especially designed for immunocytochemical applications and is based on Gill's formula, an alcohol-free solution containing no mercury. This formulation is also ideally suited for sections developed with alcohol-soluble enzyme reaction products, such as AEC, ImmPACT™ AEC or ImmPACT™ AMEC Red.

Vector® Hematoxylin QS	H-3404	100 ml
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Vector® Hematoxylin QS, a modification of Mayer's hematoxylin developed especially for immunocytochemistry, provides crisp blue-violet nuclear staining without obscuring antigen-specific chromogen deposition. Vector® Hematoxylin QS requires no “blueing” step, stains in less than 45 seconds, contains no mercury, and is ready-to-use without filtration.

Vector® Methyl Green	H-3402	500 ml
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Methyl Green can be used with a wide range of enzyme reaction products and is especially suited for multiple label applications. It is also ideal for black and white photography of immunohistochemically stained sections. Our improved formulation of this counterstain allows sections to be stained optimally using a simple, two-step protocol.

Vector® Nuclear Fast Red	H-3403	500 ml
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Nuclear Fast Red stains nuclei pink to red. Tissue sections can be counterstained in a rapid, one-step protocol.

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Detailed product listings, specifications and protocols are available on our website:

www.vectorlabs.com

The Vector® M.O.M.™ ImmPRESS™ Kit is designed for laboratory use only.