



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 monoclonal and polyclonal 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 endogenous mouse immunoglobulins in the tissue. A consequence of this problem has been 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 should be helpful when working 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 Ig 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.

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

- M.O.M.™ Mouse Ig 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 ml of 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.

ENZYME SUBSTRATES

A variety of chromogens can be used to localize peroxidase substrates in tissue sections. Vector Laboratories offers the traditional substrates DAB and AEC as well as several proprietary substrates, producing colors as listed.

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

DAB (Diaminobenzidine), SK-4100, brown
DAB + Ni²⁺, SK-4100, gray/black
Vector® VIP, SK-4600, purple
Vector® SG, SK-4700, blue-gray
Vector® NovaRED™, SK-4800, dark red
TMB, SK-4400, blue
AEC (3-amino-9-ethyl carbazole)*, SK-4200, red

Vector Laboratories also offers a line of peroxidase substrates with increased sensitivity.

ImmPACT™ DAB, SK-4105, brown
ImmPACT™ VIP, SK-4605, purple
ImmPACT™ SG, SK-4705, blue-gray
ImmPACT™ NovaRED™, SK-4805, dark red
ImmPACT™ AEC*, SK-4205, red

* AEC is 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.

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 peroxidase activity if required*:
 - 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 for 5 minutes.
 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 Ig 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® *Elite* 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. See note 4 for approximate development times.
- * 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.

NOTES:

1. The amount of endogenous immunoglobulin will vary by tissue type, fixation, and a variety of other factors. This kit should be optimized for individual application. In some cases, decreasing the concentration of M.O.M.™ Biotinylated Anti-Mouse IgG Reagent, slightly increasing or decreasing the concentration of M.O.M.™ Mouse Ig Blocking Reagent, or lengthening the incubation (step 7) in M.O.M.™ Mouse Ig Blocking Reagent can enhance the kit's performance. (See Vector® Troubleshooting Guide: Mouse Antibodies on Mouse Tissues.)
2. Not all background present in a tissue section will be caused by endogenous mouse Ig. Appropriate negative control sections should be run in parallel, to rule out other possible causes of background. (See Vector® Troubleshooting Guide.)
3. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in preparing the peroxidase substrate or added to the VECTASTAIN® *Elite* ABC Reagent. Do not add normal serum, non-fat dried milk, culture media or other potential sources of biotin to this reagent. This may result in reduced sensitivity.
4. Development times may differ depending upon the level of antigen, the intensity of the stain that is required or the substrate used. DAB generally should be developed for 2-10 minutes; Vector® VIP for 2-15 minutes; Vector® SG for 2-10 minutes; Vector® NovaRED™ for 2-15 minutes; TMB for 5-10 minutes; AEC for 10-30 minutes. Some counterstains may not be compatible with certain peroxidase substrates because of solubility of the reaction products or lack of color contrast. Refer to the instructions in the respective substrate kits for further details.
5. In the presence of nickel ions, the precipitate formed by DAB is gray/black rather than brown. The Vector® DAB Substrate Kit (Cat. No. SK-4100) contains nickel chloride, allowing a choice of colors for development.
6. The section should be well prepared. Fixation (generally, in buffered formalin not exceeding 4% formaldehyde) should be sufficient to maintain the integrity of the section throughout the staining procedure but not so harsh as to destroy the antigen under study. If staining is absent, unmasking of antigens may be required before the primary antibody can bind. Special Antigen Unmasking Solutions (Cat. No. H-3300 or H-3301) and a detailed protocol describing the method are available. During the staining procedure, do not allow the section to dry out. If necessary, use a humidified chamber for incubations.

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7. 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 the substrate solution be prepared with glass distilled water. Deionized water (even with low conductivities) may contain inhibitors of peroxidase and can reduce sensitivity.
8. The Vector® M.O.M.™ Kit should be stored under refrigeration. For best results, the reagents should be used before the date shown on the box. We recommend that they 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.
9. Sections of neuronal tissue or sections which are thicker than normal may require longer incubation times for optimal staining.
10. Specimens should not be embedded in paraffin heated higher than 60 °C. Too much heat can destroy antigens. After mounting, paraffin sections should be dried in a hot air oven at 50-56 °C. Some slide warmers contain “hot spots” that can overheat tissues. Complete deparaffinization is important. Clearing agents and alcohol solutions should be changed regularly. All steps of the deparaffinization should be sufficiently long to completely remove the paraffin from the sections.
11. Paraffin tissue blocks should be stored in sealed containers in a cool location.
12. 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. Do not use egg albumin coated slides. Traces of egg white avidin may affect staining quality.
13. Hand lotions can cause sections to detach from slides or may prevent adequate penetration of reagents. Avoid touching rinse baths with oily hands.
14. Not all mouse monoclonal and polyclonal antibodies recognize antigens of mouse origin. The species cross-reactivity of a given mouse primary antibody should be established to avoid false negative results.

PEROXIDASE SUBSTRATE KITS

These peroxidase substrates are available as concentrated stock solutions in two different dropper-bottle formats, the classic kits that provide 300 ml of working solution or the more sensitive, new formula versions (ImmPACT™) sufficient to prepare 120 ml of working solution. These substrates produce insoluble colored reaction products ideal for immunohistochemical applications.

DAB (brown/black)

- **ImmPACT™ DAB** SK-4105 • 120 ml
 - **DAB Substrate Kit** SK-4100 • 1 kit
- Vector® DAB Substrate Kit produces a brown, dense precipitate which can be used alone or in combination with other substrates in multiple labeling protocols. Vector® DAB stained sections can be dehydrated and permanently mounted.

DAB Substrate Kit, SK-4100, contains a separate nickel solution to provide the color option of a gray/black color.

- **DAB Enhancing Solution** H-2200 • 30 ml
- DAB Enhancing Solution is useful for intensifying the reaction product in sections stained with DAB. A ten second exposure to this solution enhances the DAB reaction product, provides a moderate increase in sensitivity, and can reduce decolorizing during dehydration and permanent mounting.

NovaRED™ (dark red)

- **ImmPACT™ NovaRED™** SK-4805 • 120 ml
 - **VECTOR® NovaRED™ Substrate Kit** SK-4800 • 1 kit
- Vector® NovaRED™ Substrate Kit produces a red, dense precipitate which can be used alone or in combination with other substrates in multiple labeling protocols. Vector® NovaRED™ stained sections can be dehydrated and permanently mounted. Sections stained with this substrate can also be viewed by darkfield microscopy.

VIP (purple)

- **ImmPACT™ VIP** SK-4605 • 120 ml
 - **VECTOR® VIP Substrate Kit** SK-4600 • 1 kit
- Vector® VIP Substrate Kit produces an intense, violet-colored precipitate useful as an alternative substrate to DAB or as a second color for multiple label immunohistochemical staining. Vector® VIP stained sections can be dehydrated and permanently mounted. Sections stained with this substrate can also be viewed by darkfield microscopy. This product can also be employed for electron microscopy.

SG (blue-grey)

- **ImmPACT™ SG** SK-4705 • 120 ml
 - **VECTOR® SG Substrate Kit** SK-4700 • 1 kit
- Vector® SG Substrate Kit produces a bluish-gray reaction product. This substrate can be used singly or as a second label in peroxidase staining systems. Sections developed with this substrate can be dehydrated and permanently mounted. Sections stained with this substrate can also be viewed by darkfield microscopy. This product can also be employed for electron microscopy.

AEC (red)

- **ImmPACT™ AEC** SK-4205 • 1 kit
 - **AEC Substrate Kit** SK-4200 • 1 kit
- 3-amino-9-ethylcarbazole
AEC Substrate Kit produces a red to brown reaction product. Sections developed with AEC must be aqueously mounted.

TMB (blue)

- **TMB Substrate Kit** SK-4400 • 1 kit
- The TMB Kit produces a blue reaction product that is very sensitive for immunohistochemistry and *in situ* hybridization. TMB stained sections can be dehydrated and permanently mounted.

ADDITIONAL REAGENTS

- **VECTABOND™ Reagent** SP-1800 • 7 ml
- VECTABOND™ Reagent is a novel tissue section adhesive that can 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
- This hydrophobic barrier pen is lightly colored to be seen during and after application. The ImmEdge™ Pen keeps reagents localized to tissue sections, remains through all aqueous steps, is economical, and ideal for differentially staining two sections on the same slide.

- **ImmPrint™ Histology Pen** H-6100 • 5-pen set
- 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.

- **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 of biotin in convenient dropper bottles. These kits are designed for use in those cases when streptavidin, avidin, or biotinylated products bind nonspecifically to tissues or proteins.

- **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. The Antigen Unmasking Solution is supplied as an approximately 100x concentrated stock sufficient to prepare 25 liters of working solution. A detailed protocol describing optimal conditions for use is included.

- **VectaMount™ Mounting Medium** H-5000 • 60 ml
- 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
- This aqueous hard-setting mounting medium is designed for use with enzyme substrates, such as AEC, whose reaction products are soluble in alcohol or other organic solvents.

ADDITIONAL VECTOR® M.O.M.™ KITS

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

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

- **Mouse Ig Blocking Reagent** MKB-2213 • 1 ml
- This reagent is the same as that contained in the M.O.M.™ kits.

- **Biotinylated Anti-Mouse IgG Reagent** MKB-2225 • 0.1 ml
- This reagent is the same as that contained in the M.O.M.™ kits.

COUNTERSTAINS

- **Vector® Hematoxylin** H-3401 • 500 ml
- 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.

- **Hematoxylin QS** H-3404 • 100 ml
- 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. Requiring no “blueing” step and less than 45 seconds to stain, Vector® Hematoxylin QS contains no mercury and is ready-to-use without filtration.

- **Vector® Methyl Green** H-3402 • 500 ml
- 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
- 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.™ Kit is designed to be used for laboratory use only.