



COMPONENTS

The Vector® M.O.M.™ Kit with ImmPRESS™ Peroxidase Polymer contains:

- 0.6 ml concentrated stock solution of M.O.M.™ Mouse IgG Blocking Reagent
- 30 ml ready-to-use (R.T.U.) 2.5% Normal Horse Serum (NHS) for general protein blocking
- 15 ml R.T.U. M.O.M.™ ImmPRESS™ Anti-Mouse IgG Reagent (made in horse, ready-to-use)

The Vector® M.O.M.™ ImmPRESS™ Immunodetection Kit contains enough reagents to stain approximately 75-150 mouse sections.

PREPARATION OF M.O.M.™ MOUSE IgG BLOCKING SOLUTION

- M.O.M.™ Mouse IgG Blocking Reagent: add 2 drops of stock solution to 2.5 ml of PBS or TBS. †

◇ 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

M.O.M.™ KIT STAINING PROCEDURE for Paraffin Sections

1. For paraffin sections, deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.

For frozen sections or cell preparations, fix with acetone or an appropriate fixative for the antigen under study. Air dry.

Rinse for 5 minutes in tap water.

2. If antigen unmasking is required, perform this procedure using a Vector® Antigen Unmasking Solution, Citrate-based (H-3300) or High pH-based (H-3301).

3. Block endogenous enzyme activity, if necessary, by incubating sections with BLOXALL™ Blocking Solution (SP-6000) for 10 minutes.* For alternative blocking protocols see Note 4.

4. Wash 2 x 2 minutes in PBS or TBS.

5. Incubate for 1 hour in working solution of M.O.M.™ Mouse IgG Blocking Reagent prepared as described.

6. Wash 2 x 2 minutes in PBS or TBS.**

7. Incubate for 5 minutes in R.T.U. 2.5% Normal Horse Serum (NHS).**

8. Tip off excess NHS. Dilute primary antibody in R.T.U. 2.5% NHS. Incubate section in diluted primary antibody for 30 minutes.**

9. Wash for 2 x 2 minutes in PBS or TBS.**

10. Apply the Vector® M.O.M.™ ImmPRESS™ Reagent and incubate for 10 minutes.**

11. Wash for 2 x 5 minutes in PBS or TBS.

12. Prepare and apply peroxidase substrate solution according to substrate kit instructions.

* When appropriate control sections have shown that endogenous enzyme activity is not present, step 3 may be omitted.

** It is recommended that the exact times described in steps 6-10 be used in the staining protocol.

CUSTOMIZATION OF M.O.M.™ KIT PROTOCOL

Off-target binding, at least in part, can be due to factors other than endogenous mouse IgG such as 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.™ ImmPRESS™ 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: www.vectorlabs.com

Vector® M.O.M.™ Kit ImmPRESS™ Peroxidase Polymer

Catalog No. MP-2400

Introduction

The Vector® M.O.M.™ Immunodetection Kits are specifically designed to localize mouse primary antibodies on mouse tissues. The major problem with using mouse primary antibodies on mouse tissues in immunohistochemistry is the inability of the anti-mouse secondary antibody to distinguish between the mouse primary antibody and endogenous mouse immunoglobulins in the tissue. This can result in high background staining which obscures specific staining. This problem can be essentially eliminated by using a Vector® M.O.M.™ Immunodetection Kit which includes a proprietary M.O.M.™ Mouse IgG Blocking Reagent with a specialized ImmPRESS™ Anti-Mouse IgG to significantly reduce undesired binding of the secondary antibody to endogenous tissue immunoglobulin. The ImmPRESS™ Reagent uses novel conjugation and micropolymer chemistries to create a highly sensitive, ready-to-use, one-step, non-biotin detection system. The unique peroxidase micropolymers of the ImmPRESS™ reagent limit steric interference and provide enhanced accessibility to the target. The result is crisp, strong staining of antibody targets, especially nuclear and membrane antigens.

NOTES:

1. The Vector® M.O.M.™ ImmPRESS™ 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. 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.
3. Thicker sections may require longer incubation times for optimal staining. Appropriate control slides should be run in parallel if incubation times are altered.
4. 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.

5. Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining.
6. To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (SP-1800), a non-protein tissue section adhesive.

PEROXIDASE SUBSTRATES

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

Note: A chart of the Relative Sensitivity of Substrates in Immunohistochemistry is available on our website: www.vectorlabs.com

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

Vector® M.O.M.™ Mouse IgG Blocking Reagent MKB-2213 1 ml

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

This product contains the same reagent as that included in the M.O.M.™ kits.

Vector® M.O.M.™ Basic Kit BMK-2202 1 kit

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.™ Peroxidase Immunodetection Kit PK-2200 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 the VECTASTAIN® *Elite* ABC Reagents.

ADDITIONAL REAGENTS FOR VECTOR® M.O.M.™ IMPRESS™ KITS

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 0.45µm filter.

BLOXALL™ Blocking Solution SP-6000 100 ml

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.

VECTABOND™ Reagent SP-1800 7 ml

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

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

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. The Antigen Unmasking Solution is supplied as an approximately 100x concentrated stock sufficient to prepare 25 liters of working solution.

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, ImmPACT™ AEC or ImmPACT™ AMEC Red, whose reaction products are soluble in alcohol or other organic solvents.

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, ImmPACT™ AEC or ImmPACT™ AMEC Red.

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