



VECTOR® M.O.M.™ Immunodetection Kit

BASIC

Catalog No. BMK-2202

Introduction

Vector® M.O.M.™ Immunodetection Kits are 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 Vector® M.O.M.™ Immunodetection Kits which utilize a novel blocking agent and special detection methodology to significantly reduce this undesired background staining.

Vector® M.O.M.™ Immunodetection Kits are offered in two formats with the detection system provided: the peroxidase (PK-2200) kit which includes VECTASTAIN® ABC Reagents and the fluorescent kit (FMK-2201) which includes Fluorescein Avidin DCS. The Vector® M.O.M.™ Basic Kit does not include a detection system, enabling the user to incorporate the detection reagent of choice.

The Vector® M.O.M.™ Basic Kit provides the flexibility of using any avidin- or streptavidin-based detection system, employing either fluorescent or enzyme labels for visualization of the target antigen. In addition, the M.O.M.™ Basic Kit is ideal for localizing multiple antigens present in the same mouse tissue section using our suggested multiple antigen labeling protocols.

COMPONENTS

The Vector® M.O.M.™ Basic 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

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.

Note: This Vector® M.O.M.™ Basic Kit is to be used with an avidin- or streptavidin-based detection system (not included). A number of different enzyme or fluorescent systems can be utilized with the Vector® M.O.M.™ Basic Kit (see "Suggested Detection Systems").

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

- M.O.M.™ Mouse Ig Blocking Reagent: add 2 drops \diamond of stock solution to 2.5 ml of PBS or TBS. \uparrow
- M.O.M.™ Diluent: add 600 μ l of Protein Concentrate stock solution to 7.5 ml of PBS or TBS. $\uparrow\uparrow$
- M.O.M.™ Biotinylated Anti-Mouse IgG Reagent: add 10 μ l of stock solution to 2.5 ml of M.O.M.™ diluent prepared above.

\diamond One drop is approximately 45 μ l

\uparrow PBS: 10mM sodium phosphate, 0.15M NaCl, pH 7.4-7.8
TBS: 50mM TRIS, 0.15M NaCl, pH 7.5-7.8

$\uparrow\uparrow$ Note: 7.5 ml of M.O.M.™ diluent provides sufficient reagent for use in steps 9, 10, and 12.

BLOCKING ENDOGENOUS ACTIVITY

If a peroxidase- or alkaline phosphatase-based system is used for detection, one of the following procedures may be required to destroy endogenous enzyme activity prior to using the M.O.M.™ Basic Kit.

To block endogenous peroxidase activity:

- 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.

To block endogenous alkaline phosphatase activity:

Endogenous alkaline phosphatase activity is less common in paraffin sections than in frozen sections and is generally completely absent in sections treated with high temperature to unmask antigens. If the endogenous activity is an isoenzyme other than the intestinal form, it can be inhibited by the addition of levamisole (Cat. No. SP-5000) to the buffer used to prepare the substrate solution. Intestinal alkaline phosphatase can be inhibited by treating the sections, prior to staining, either with 20% acetic acid at 4 °C for 15 seconds or with 2.3% periodic acid for 5 minutes and 0.02% potassium borohydride for 2 minutes.

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

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 or H-3301.)
4. Block endogenous enzyme activity if necessary.*
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) or Vector® Streptavidin/Biotin Blocking Kit (Cat. No. SP-2002).
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 the appropriate avidin- or streptavidin-based detection system (see "Suggested Detection Systems"). *For enzymatic detection see "Enzyme Detection"; for fluorescence see "Fluorescent Detection".*

* When appropriate control sections have shown that endogenous enzyme 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.

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

1. Fix sections in acetone or appropriate fixative for antigen under study. *See note 4.*
2. Air dry sections.
3. Wash section 2 x 2 minutes in PBS or TBS.
4. Continue from step 4 of the staining procedure for Paraffin Sections.

ENZYME DETECTION

Enzyme conjugated avidin or streptavidin or a VECTASTAIN® ABC preformed complex would be used in step 14 of the staining procedure. After incubation for the time suggested for the conjugate or complex, wash the section 2 x 2 minutes in PBS or TBS followed by the appropriate enzyme substrate. Refer to the substrate kit instructions for development times and mounting suggestions. *See note 12.*

FLUORESCENT DETECTION

In step 14, use the suggested concentration (generally 15-30 μ g/ml) and incubation times (generally 5 minutes) for the fluorochrome conjugated avidin or streptavidin. Wash section for 2 x 5 minutes in PBS or TBS then mount section in a suitable medium such as VECTASHIELD® Mounting Medium (See product listing). *See note 13.*

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 (also available separately as Cat. No. MKB-2225), slightly increasing or decreasing the concentration of M.O.M.™ Mouse Ig Blocking Reagent (also available separately as Cat. No. MKB-2213), 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 detection systems or their substrates. This may result in reduced sensitivity.
4. Aldehyde-fixed tissue (e.g. formalin) tends to be autofluorescent and may make interpretation of specific fluorescein signal difficult.
5. The section should be well prepared. Fixation 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 in formalin-fixed tissue, unmasking of antigens may be required before the primary antibody can bind. 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.

Vector Laboratories, Inc.

30 Ingold Road • Burlingame, CA 94010
Tel: (650) 697-3600 • Fax: (650) 697-0339
E-mail: vector@vectorlabs.com
Website: www.vectorlabs.com

- 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 enzyme substrate solutions be prepared with glass distilled water. Deionized water (even with low conductivities) may contain enzyme inhibitors of peroxidase and can reduce sensitivity.
- 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.
- 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.
- 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.
- Hand lotions can cause sections to detach from slides or may prevent adequate penetration of reagents. Avoid touching rinse baths with oily hands.
- 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.
- VECTASTAIN® *Elite*® ABC Reagent incubation times can be shortened to 5-10 minutes by forming the ABC complex as follows: add 2 drops Reagent A to 2.5 mls 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.
- If a fluorescent detection system is chosen, the sensitivity of avidin- or streptavidin-conjugated fluorescent detection systems can be increased by using biotinylated Anti-Avidin (BA-0300) or biotinylated Anti-Streptavidin (BA-0500) respectively, after step 14, followed by re-application of the avidin- or streptavidin-conjugated fluorochrome.
- The 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.

SUGGESTED DETECTION SYSTEMS

The Vector® M.O.M.™ Basic Immunodetection Kit allows the use of many different avidin- or streptavidin-based detection systems. The listing below includes only a few of the possible reagents that can be used with the kit.

ENZYMATIC DETECTION SYSTEMS

• PEROXIDASE			
VECTASTAIN® Elite ABC			
Standard Kit	PK-6100	1 kit	
R.T.U. VECTASTAIN® Elite			
ABC Reagent†	PK-7100	50 ml	
Horseradish Peroxidase Avidin D	A-2004	5 mg	
R.T.U. Horseradish Peroxidase			
Avidin D†	A-2704	100 ml	
Horseradish Peroxidase			
Streptavidin	SA-5004	1 mg	
R.T.U. Horseradish Peroxidase			
Streptavidin†	SA-5704	100 ml	

• PEROXIDASE SUBSTRATES			
ImmPACT™ DAB (Brown)	SK-4105	120 ml	
ImmPACT™ AEC (Red)	SK-4205	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 to 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	

• ALKALINE PHOSPHATASE			
VECTASTAIN® ABC-AP			
Standard Kit	AK-5000	1 kit	
Alkaline Phosphatase			
Streptavidin	SA-5100	1 ml	

• ALKALINE PHOSPHATASE SUBSTRATES			
Vector® Red (Red)	SK-5100	1 kit	
Vector® Blue (Blue)	SK-5300	1 kit	
BCIP/NBT (Blue)	SK-5400	1 kit	
Vector® Black (Black)	SK-5200	1 kit	

FLUOROCROME DETECTION SYSTEMS

Fluorescein Avidin DCS	A-2011	1 mg	
Texas Red® Avidin DCS	A-2016	1 mg	
DyLight® 488 Streptavidin	SA-5488	1 mg	
DyLight® 549 Streptavidin	SA-5549	1 mg	
DyLight® 594 Streptavidin	SA-5594	1 mg	
DyLight® 649 Streptavidin	SA-5649	1 mg	
Fluorescein Streptavidin	SA-5001	1 mg	
Texas Red® Streptavidin	SA-5006	1 mg	
Biotinylated Anti-Avidin D††	BA-0300	0.5 mg	
made in goat			
Biotinylated Anti-Streptavidin††	BA-0500	0.5 mg	
made in goat			

† R.T.U. reagents and kits are provided in prediluted, ready-to-use form.

†† These products can be used to amplify the fluorescent signal of fluorescent avidin conjugates or fluorescent streptavidin conjugates, respectively. See Note 13.

ADDITIONAL REAGENTS

- M.O.M.™ Mouse Ig Blocking Reagent** MKB-2213 • 1 ml

This reagent is the same as that contained in the M.O.M.™ kits.

- M.O.M.™ Biotinylated Anti-Mouse IgG Reagent** MKB-2225 • 0.1 ml

This reagent is the same as that contained in the M.O.M.™ kits.

- 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.

- VECTASHIELD® Mounting Medium**
- | | | |
|-----------------------|--------|---------|
| | H-1000 | • 10 ml |
| with DAPI | H-1200 | • 10 ml |
| with Propidium Iodide | H-1300 | • 10 ml |

- VECTASHIELD® Hard+Set™ Mounting Medium**
- | | | |
|-----------|--------|---------|
| | H-1400 | • 10 ml |
| with DAPI | H-1500 | • 10 ml |

These unique formulas significantly reduce photobleaching of fluorescently labeled sections. VECTASHIELD® Mounting Medium has an ideal refractive index, provides strong initial fluorescence, retards photobleaching during illumination, and preserves the fluorescent signal on storage. VECTASHIELD® Hard+Set™ has all the properties of VECTASHIELD® but it also hardens.

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.

- 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.™ Kit is designed to be used for laboratory use only.