Vector® M.O.M.™ Immunodetection Kit

BASIC

Catalog No. BMK-2202

Introduction

The Vector® M.O.M.™ Immunodetection Kit is designed specifically to localize mouse primary antibodies on mouse tissues. The major problem with using 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. This can result in high background staining which obscures specific staining.

The background staining caused by the presence of endogenous mouse IgG can be essentially eliminated by using the Vector® M.O.M.™ Immunodetection Kit. The M.O.M.™ Kit utilizes a novel blocking agent and a 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.

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COMPONENTS

Reagents supplied:

- 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

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.

Reagents not supplied:

The Vector® M.O.M.™ Basic Kit is designed 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", on reverse).

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

- M.O.M.™ Mouse IgG Blocking Reagent: add 2 drops (90 µl) 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.

† PBS: 10 mM sodium phosphate, 0.15 M NaCl, pH 7.4-7.8
TBS: 50 mM TRIS, 0.15 M NaCl, pH 7.5-7.8
†† Note: 7.5 ml of M.O.M.™ Diluent provides sufficient reagent for use in steps 8, 9, and 11.

M.O.M.™ KIT STAINING PROCEDURE

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. Perform Avidin/Biotin blocking if required*, using Vector® Avidin/Biotin Blocking Kit (SP-2001) or Vector® Streptavidin/Biotin Blocking Kit (SP-2002).

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

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

8. Incubate for 5 minutes in working solution of M.O.M.™ Diluent prepared as described**.

9. 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**.

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

11. Apply working solution of M.O.M.™ Biotinylated Anti-Mouse IgG Reagent prepared as described. Incubate sections for 10 minutes**.

12. Wash sections for 2 x 2 minutes in PBS or TBS.

13. Apply the appropriate avidin- or streptavidin-based detection system (see "Suggested Detection Systems", on revese).

* When appropriate control sections have shown that endogenous enzyme or endogenous avidin/biotin activity is not present, step 3 and/or step 5 may be omitted.

** It is recommended that the exact times described in steps 7-11 be used in the staining protocol. Longer incubation may result in an increase in background staining.

CHROMOGENIC DETECTION

An enzyme conjugated avidin or streptavidin or a VECTASTAIN® ABC complex can be used in step 13 of the staining procedure. After incubation for the time suggested for the specific conjugate or complex, wash sections 2 x 5 minutes in PBS or TBS and follow with the appropriate enzyme substrate. Refer to the substrate kit instructions for development times and mounting suggestions.

FLUORESCENT DETECTION

If a fluorochrome conjugated avidin or streptavidin is used in step 13 of the staining protocol, refer to the conjugate's instructions for recommended concentration and incubation time. Wash section for 2 x 5 minutes in PBS or TBS and mount sections in suitable medium such as VECTASHIELD® Mounting Medium (see product listing).

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.™ 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: www.vectorlabs.com.

NOTES:

1. 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.

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. Although BLOXALL™ is most effective for inhibiting endogenous peroxidase or alkaline phosphatase, these alternative protocols can be used:

   To block 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.

   To block endogenous alkaline phosphatase:
   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 (SP-5000) to the buffer used to prepare the substrate solution. Intestinal alkaline phosphatase can be inhibited by following procedures described in the following reference: Bulman, A.S., Heyderman, E.; J. Clin. Pathol. 34, 1349-1351, 1981.

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.

7. Aldehyde-fixed tissue (e.g. formalin) and certain endogenous cellular/tissue elements may be autofluorescent. This may make interpretation of a specific fluorescein signal difficult. Use proper controls to determine if autofluorescence is a problem. References for reducing autofluorescence are available upon request.

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 REAGENTS

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

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

• PEROXIDASE SUBSTRATES

ImmPACT™ DAB *EqV* (Brown)	400 ml	SK-4103
ImmPACT™ DAB (Brown)	120 ml	SK-4105
ImmPACT™ AEC (Red)	120 ml	SK-4205
ImmPACT™ AMEC Red (Red)	120 ml	SK-4285
ImmPACT™ VIP (Purple)	120 ml	SK-4605
ImmPACT™ SG (Blue/gray)	120 ml	SK-4705
ImmPACT™ NovaRED™ (Red)	120 ml	SK-4805
DAB (Brown or gray/black)	1 Kit	SK-4100
AEC (Red)	1 Kit	SK-4200
Vector® VIP (Purple)	1 Kit	SK-4600
Vector® SG (Blue/gray)	1 Kit	SK-4700
Vector® NovaRED™ (Red)	1 Kit	SK-4800

• ALKALINE PHOSPHATASE REAGENTS

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

• ALKALINE PHOSPHATASE SUBSTRATES

ImmPACT™ Vector® Red (Magenta)	1 Kit	SK-5105
Vector® Red (Magenta)	1 Kit	SK-5100
Vector® Blue (Blue)	1 Kit	SK-5300
BCIP/NBT (Indigo)	1 Kit	SK-5400
Vector® Black (Black)	1 Kit	SK-5200

FLUOROCHROME DETECTION SYSTEMS

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

‡‡ These products can be used to amplify the fluorescent signal of fluorescent avidin conjugates or fluorescent streptavidin conjugates, respectively.

VECTOR® M.O.M.™ KITS AND REAGENTS

Vector® M.O.M.™ Basic Kit	1 kit	BMK-2202
Vector® M.O.M.™ Fluorescein Kit	1 kit	FMK-2201
Vector® M.O.M.™ Peroxidase Immunodetection Kit	1 kit	PK-2200
M.O.M.™ Mouse IgG Blocking Reagent	1 ml	MKB-2213
M.O.M.™ Biotinylated Anti-Mouse IgG Reagent	0.1 ml	MKB-2225
Vector® M.O.M.™ ImmPRESS™ Polymer Kit	1 kit	MP-2400
Vector® M.O.M.™ ImmPRESS™ Polymer Reagent	15 ml	MPX-2402

ADDITIONAL REAGENTS

BLOXALL™ Blocking Solution	100 ml	SP-6000
Avidin/Biotin Blocking Kit	1 Kit	SP-2001
Streptavidin/Biotin Blocking Kit	1 Kit	SP-2002
VECTABOND™ Reagent	7 ml	SP-1800
ImmEdge™ Pen	2-pen set	H-4000
ImmPrint™ Histology Pen	5-pen set	H-6100
Antigen Unmasking Solution		
Citrate-based	250 ml	H-3300
High pH	250 ml	H-3301
MOUNTING MEDIA		
VectaMount™ Mounting Medium	60 ml	H-5000
VectaMount™ AQ Mounting Medium	60 ml	H-5501
VECTASHIELD® Mounting Medium	10 ml	H-1000
with DAPI	10 ml	H-1200
with Propidium Iodide	10 ml	H-1300
VECTASHIELD® Hard•Set™ Mounting Medium	10 ml	H-1400
with DAPI	10 ml	H-1500
COUNTERSTAINS		
Vector® Hematoxylin	500 ml	H-3401
Vector® Hematoxylin QS	100 ml	H-3404
Vector® Methyl Green	500 ml	H-3402
Vector® Nuclear Fast Red	500 ml	H-3403

Detailed product listings, specifications and protocols are available on our website: w**ww.vectorlabs.com**

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