



Vector® M.O.M.™ Immunodetection Kit FLUORESCHEIN Catalog No. FMK-2201

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.

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
- 0.4 ml Fluorescein Avidin DCS

Excitation maximum of fluorescein is at 495nm. Emission maximum is at 515nm.

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 (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.
- Fluorescein Avidin DCS: add 40 µl of stock solution to 2.5 ml of PBS or TBS.

† *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 7, 8, and 10.*

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.

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. Wash in buffer for 5 minutes.
 4. Perform Avidin/Biotin blocking if required*, using Vector® Avidin/Biotin Blocking Kit (SP-2001) or Vector® Streptavidin/Biotin Blocking Kit (SP-2002).
 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 tissue sections for 5 minutes in working solution of M.O.M.™ Diluent prepared as described**.
 8. 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**.
 9. Wash for 2 x 2 minutes in PBS or TBS**.
 10. Apply working solution of M.O.M.™ Biotinylated Anti-Mouse IgG Reagent prepared as described. Incubate sections for 10 minutes**.
 11. Wash for 2 x 2 minutes in PBS or TBS.
 12. Apply Fluorescein Avidin DCS prepared as described. Incubate for 5 minutes.
 13. Wash for 2 x 5 minutes in PBS or TBS.
 14. Mount in a suitable medium such as one of the VECTASHIELD® Mounting Media (H-1000, H-1200, H-1300, H-1400, or H-1500).
- * When appropriate control sections have shown that endogenous avidin/biotin activity is not present, step 4 may be omitted.
- ** It is recommended that the exact times described in steps 6-12 be used in the staining protocol. Longer incubation may result in an increase in background staining.

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. Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining.
5. The sensitivity of this kit can be increased by using Biotinylated Anti-Avidin D (BA-0300), followed by Fluorescein Avidin DCS.
6. To prevent sections from detaching from the glass, slides can be pretreated with VECTABOND™ Reagent (Cat. No. SP-1800), a non-protein tissue section adhesive, before sections are mounted.
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.

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.

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

Vector® M.O.M.™ Peroxidase Immunodetection Kit
1 kit PK-2200

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.

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

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.

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

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

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

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

VECTOR® M.O.M.™ ImmPRESS™ POLYMER KITS

Vector® M.O.M.™ ImmPRESS™ Kit 1 kit MP-2400

This peroxidase polymer kit contains M.O.M.™ Mouse IgG Blocking Reagent, R.T.U. Normal Horse Serum, and the M.O.M.™ ImmPRESS™ Anti-Mouse IgG Reagent.

Vector® M.O.M. ImmPRESS™ Reagent 15 ml MPX-2402

This product contains the same reagent as that included in the M.O.M.™ ImmPRESS™ Kit (MP-2400).

ADDITIONAL REAGENTS

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

These blocking kits consist of 18 ml of Avidin D or Streptavidin and 18 ml of biotin in convenient dropper bottles. These kits block all endogenous biotin, biotin receptors, and avidin or streptavidin binding sites present in tissues.

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

VECTASHIELD® Mounting Media are unsurpassed at preventing photobleaching. They have an ideal refractive index and reduce the photobleaching of fluorescently labeled specimens, even after prolonged storage. They are compatible with fluorescein, Texas Red®, DyLight® dyes, Alexa Fluor® dyes, AMCA, fluorescent nuclear stains, fluorescent proteins, fluorescent tracers, histochemical stains, and most other fluorochromes. VECTASHIELD® Mounting Media are available in non-hardening and hardening formulations, both with and without DAPI or propidium iodide.

VECTABOND™ Reagent 7 ml SP-1800

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 2-pen set H-4000

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

ImmPrint™ Histology Pen 5-pen set H-6100

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	250 ml	H-3300
High pH	250 ml	H-3301

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.

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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 for laboratory use only.