



VECTOR® M.O.M.™ Immunodetection Kit

FLUORESCCEIN

Catalog No. FMK-2201

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.

COMPONENTS

The Vector® M.O.M.™ Immunodetection Kit (Fluorescein) 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
- 0.4 ml Fluorescein Avidin DCS

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.
- Fluorescein Avidin DCS: add 40 µl 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

†† Note: 7.5 ml of M.O.M.™ diluent provides sufficient reagent for use in steps 7, 8, and 10.

FLUORESCCEIN M.O.M.™ KIT STAINING PROCEDURE FOR FROZEN SECTIONS

1. Fix sections in acetone or appropriate fixative for antigen under study. *See Note 3*
2. Air dry sections.
3. Wash section 2 x 2 minutes in PBS or TBS.
4. Perform Avidin/Biotin blocking if required*, using Vector® Avidin/Biotin Blocking Kit (Cat. No. SP-2001).
5. Incubate sections for 1 hour in working solution of M.O.M.™ Mouse Ig Blocking Reagent prepared as described.
6. Wash sections 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 sections 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 sections for 2 x 2 minutes in PBS or TBS.
12. Apply Fluorescein Avidin DCS prepared as described. Incubate sections for 5 minutes.
13. Wash sections for 2 x 5 minutes in PBS or TBS.
14. Mount sections in a suitable medium such as VECTASHIELD® Mounting Medium (H-1000).

* 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 6-10 be used in the staining protocol. Longer incubation may result in an increase in background staining. If an extended incubation time is used, appropriate negative controls should be run in parallel to ensure the efficacy of the M.O.M.™ Mouse Ig Blocking Reagent.

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 (also available separately as Cat. No. MKB-2213), or lengthening the incubation (step 5) in M.O.M.™ Mouse Ig Blocking Reagent can enhance the kit's performance. (See Trouble-shooting: Mouse Antibodies on Mouse Tissue.)
2. Not all background present in a tissue section will be caused by endogenous mouse IgG. Appropriate negative control sections should be run in parallel to rule out other possible causes of background. (See Vector® Troubleshooting Guide.)
3. Aldehyde-fixed tissue (e.g. formalin) tends to be autofluorescent and may make interpretation of specific fluorescein signal difficult.
4. 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.
5. 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.

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6. The sensitivity of this kit can be increased by using Biotinylated Anti-Avidin D (Cat. No. BA-0300), followed by Fluorescein Avidin DCS.
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.
8. 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. During the staining procedure, do not allow the section to dry out. If necessary, use a humidified chamber for incubations.
9. 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. Vector® M.O.M.™ Immunodetection Kits are also available with a peroxidase detection system (Cat. No. PK-2200) or without any enzyme or fluorochrome avidin/streptavidin conjugate (Vector® M.O.M.™ Basic Kit, Cat. No. BMK-2202).
15. 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.

ADDITIONAL REAGENTS

- **Mouse Ig Blocking Reagent** MKB-2213 • 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. 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, and is economical. 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.

- **Texas Red® Avidin DCS** A-2016 • 1 mg
- **Texas Red® Streptavidin** SA-5006 • 1 mg

These fluorochrome labeled products emit a bright red fluorescence and can be used instead of Fluorescein Avidin DCS or in conjunction with Fluorescein Avidin DCS in double labeling protocols.

- **Biotinylated Anti-Avidin D** BA-0300 • 0.5 mg
made in goat
- **Biotinylated Anti-Streptavidin** BA-0500 • 0.5 mg
made in goat

These products can be used to amplify the fluorescent signal of Fluorescein Avidin DCS and Texas Red® Avidin DCS, or Fluorescein Streptavidin and Texas Red® Streptavidin, respectively.

- **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.

ADDITIONAL VECTOR® M.O.M.™ KITS

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

This kit consists of the same basic components as the Fluorescein M.O.M.™ Kit except, in place of the Fluorescein Avidin DCS, it contains the VECTASTAIN® *Elite* ABC Reagents.

- **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.

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A comprehensive catalog of antibodies and other immunohistochemical products is available upon request or visit our website:

www.vectorlabs.com

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