TROUBLESHOOTING GUIDE

Vector® M.O.M.® (Mouse on Mouse) Immunodetection Kits.

Together we breakthrough™

Mouse antibodies on mouse tissue

Localizing a mouse primary antibody on mouse tissue using an anti-mouse immunoglobulin (Ig) detection system can produce nonspecific staining due to the presence of endogenous mouse Ig.

M.O.M. (Mouse on Mouse) Kits and M.O.M. Reagents localize mouse monoclonal and polyclonal primary antibodies on tissues of mouse origin and are specifically formulated to decrease the non-specific background due to the presence of endogenous mouse Ig. The staining procedure is simple, easy to perform, and effective on both frozen and formalin-fixed, paraffin-embedded tissues.

This M.O.M. troubleshooting guide describes some of the common difficulties encountered when using mouse antibodies on mouse tissue, and details strategies for customizing the Vector M.O.M. Kits to address them.

Vector M.O.M. Kits are available with different detection systems

Product	Catalog Number
M.O.M.® Elite® Immunodetection Kit, Peroxidase	PK-2200
M.O.M.® ImmPRESS® HRP Polymer Kit, Peroxidase	MP-2400
M.O.M.® Immunodetection Kit, Fluorescein	FMK-2201
M.O.M.® Immunodetection Kit, Basic (user supplies avidin/streptavidin-based detection system)	BMK-2202
The following are also available separately allowing the user maximum flexibility and customization.	
M.O.M.® Blocking Reagent	MKB-2213
M.O.M.® Biotinylated Anti-Mouse IgG Reagent	MKB-2225
M.O.M.® ImmPRESS Polymer Reagent, Anti-Mouse IgG, Peroxidase	MPX-2402
Normal Horse Serum Blocking Solution, 2.5%	S-2012

For technical assistance

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vector LABORATORIES

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A DETERMINE steps that contribute to background

Non-specific staining may be due, at least in part, to factors other than endogenous mouse Ig such as endogenous enzyme activity or non-specific protein interactions. Appropriate deletion controls should be done to determine the factors contributing to background staining. Some examples:

i. If staining occurs when only an enzymatic substrate is applied, endogenous enzyme activity may not be sufficiently quenched. Include an appropriate endogenous enzyme-quenching step such as BLOXALL® Endogenous Blocking Solution (SP-6000) to abolish both endogenous peroxidase and alkaline phosphatase activities.

ii. If staining occurs when only a streptavidin or avidin-based detection system and substrate are applied, endogenous biotin, or free biotin-binding sites may be present in the tissue. Include an Avidin/Biotin Blocking Kit (SP-2001) or Streptavidin/Biotin Blocking Kit (SP-2002) in the protocol.

iii. Some background staining can be due to nonspecific protein interactions. The Blocking Solutions provided in the Vector M.O.M. Kits (M.O.M. Protein Concentrate or 2.5% R.T.U Normal Horse Serum) are formulated to minimize this type of non-specific staining. In some cases, the addition of 0.1% detergent to the Blocking Solution (e.g. Tween® 20, or Triton® X-100) can improve results.

iv. If the background described in (i) through (iii) has been eliminated but non-specific staining still occurs in a control section with no primary antibody (i.e. diluent control), then the anti-mouse Ig detection system may be detecting endogenous mouse Ig in the tissue. **Section B** addresses ways to customize the M.O.M. reagents to further reduce endogenous mouse Ig detection.

v. If inappropriate staining is seen only when the mouse primary antibody or the irrelevant mouse isotype control antibody is included in the assay, then the primary antibody specificity should be evaluated and conditions optimized by adjusting the concentration and/or incubation time and temperature of the primary antibody.

These deletion controls are described in more detail in the general Troubleshooting Guides available on our website.

B CUSTOMIZE M.O.M. Kits and reagents

The amount of endogenous mouse Ig 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 Ig while maintaining high staining sensitivity.

i. The high sensitivity of Vector M.O.M. detection reagents may require customizing the dilution of the M.O.M. Biotinylated Anti-Mouse IgG Reagent or the M.O.M. ImmPRESS Anti-Mouse IgG Reagent for tissues containing especially high levels of endogenous mouse Ig.

M.O.M. Biotinylated Anti-Mouse Ig Reagent

Decrease the concentration of this reagent. For example, using only 7.0 µl in 2.5 ml M.O.M. Diluent (a 30% decrease in concentration) will result in lower endogenous mouse Ig staining with only a slight decrease in specific staining intensity.

The M.O.M. Biotinylated Anti-Mouse Ig Reagent can be further diluted, if necessary, while titrating the concentration of the primary antibody for optimized results.

M.O.M. ImmPRESS Anti-Mouse Ig Reagent

Decrease the concentration of this reagent by dilution in either PBS or 2.5% Normal Horse Serum/PBS. For example, using this reagent at 50% of its concentration will result in lower endogenous mouse Ig staining with only a slight decrease in specific staining intensity.

The M.O.M. ImmPRESS Anti-Mouse IgG Reagent can be further diluted, if necessary, while titrating the concentration of the primary antibody for optimized results.

ii. The concentration and/or the incubation time of the M.O.M. Mouse Ig Blocking Reagent can also be modified.

M.O.M. Mouse IgG Blocking Reagent

The concentration can be increased by using 3 or 4 drops in 2.5 ml PBS or decreased by using 2 drops in 5 ml PBS. Alternatively, the incubation time may be increased from one to two hours. Several users have reported using this reagent at a concentration of 5 drops in 2.5 ml overnight at 4°C, followed by 30 minutes at room temperature the following day to improve their results.