



TROUBLESHOOTING PROBLEMS ENCOUNTERED USING MOUSE ANTIBODIES ON MOUSE TISSUE

1) Determine if background staining is due to endogenous mouse immunoglobulin:

Immunohistochemical staining which localizes a mouse primary antibody on mouse tissue using an anti-mouse immunoglobulin (IgG) secondary antibody as part of the detection system can produce non-specific staining due to the presence of *endogenous mouse immunoglobulin*. The presence of endogenous mouse Ig can be confirmed by comparing the staining patterns seen with the following controls:

CONTROL SLIDES	A	B
Quench Endogenous Enzyme (if necessary)	Yes	Yes
Avidin/Biotin Blocking Step (if necessary)	Yes	Yes
Mouse Primary Antibody	No	No
Anti-Mouse IgG Secondary Antibody	No	Yes
Detection System (Enzymatic/Fluorescent)	Yes	Yes
Enzyme Substrate (if necessary)	Yes	Yes

If non-specific staining is present only in control section B, or if it is much greater than in control section A, then the secondary antibody is most likely binding to endogenous mouse Ig in the tissue.

Vector® M.O.M.™ (Mouse On Mouse) Kits are designed to localize mouse monoclonal and polyclonal primary antibodies on tissues of mouse origin. The staining procedure is simple, easy to perform, and effective on both frozen and formalin-fixed, paraffin-embedded tissues.

There are two components of the Vector® M.O.M.™ Kit that are specifically formulated to decrease the background staining due to the presence of endogenous mouse Ig:

M.O.M.™ Mouse Ig Blocking Reagent

M.O.M.™ Biotinylated Anti-Mouse IgG Reagent.

Vector® M.O.M.™ Kits are available either with a detection system, peroxidase (Cat. No. PK-2200) or fluorescein (Cat. No. FMK-2201), or as a basic kit (BMK-2202) where the user supplies the avidin/streptavidin based detection system optimal for a particular application.

2) Background staining that is not due to endogenous mouse immunoglobulin:

Some non-specific staining may be due, at least in part, to factors other than endogenous mouse Ig. Appropriate substitution controls should be done to determine which reagent(s) may be contributing to the background staining. Based on the results of these controls, the following steps may be required.

- (i) Include an avidin/biotin blocking step (Cat. No. SP-2001) in the procedure.
- (ii) If an enzymatic detection system is used, include the appropriate endogenous enzyme quenching step.

If inappropriate staining is seen only when the primary antibody is present, then the primary antibody specificity and/or its working dilution should be questioned. Each new application and tissue type should be evaluated and the protocol adjusted accordingly. Refer to the Vector® Troubleshooting Guide for more detailed information.

3) Possible modifications to the Vector® M.O.M.™ Kit:

- (i) M.O.M.™ Protein Concentrate: Although the M.O.M.™ Protein Concentrate is specially formulated to minimize background staining due to non-specific protein interactions, some inappropriate staining may still be present. In some cases, the addition of 0.1% detergent (Tween 20, Triton X-100) to the working solution of the Protein Concentrate may improve results.

For the majority of mouse tissues, the dilution and incubation times recommended in the Vector® M.O.M.™ protocol are highly effective in reducing the background caused by endogenous mouse Ig. In the event that some endogenous mouse Ig is still being detected, it may be necessary to adjust the M.O.M.™ Mouse Ig Blocking Reagent and/or the M.O.M.™ Biotinylated Anti-Mouse IgG Reagent for a particular application.

- (ii) M.O.M.™ Mouse Ig Blocking Reagent: Some modifications may be made in the concentration and/or the incubation time of this reagent. In a few cases, less reagent may be more effective. Instead of diluting this reagent 2 drops in 2.5 ml of buffer, a reduction in background staining may result from changing the dilution to 2 drops in 5 ml. In other cases, increasing the concentration of this reagent (3 or 4 drops in 2.5 ml) may be more effective. Alternatively, the blocking time may be increased. Using a 1:10 dilution (5 drops in 2.5 ml) overnight at 4 °C followed by 30 minutes at room temperature may be beneficial.
- (iii) M.O.M.™ Biotinylated Anti-Mouse IgG Reagent: This reagent may be diluted 7.0 µl in 2.5 ml. In some sections, this decrease in concentration may result in lower background staining with only a slight decrease in specific staining intensity.

NOTE: The recommended incubation times for primary antibody, biotinylated anti-mouse Ig and washes should be followed for maximal M.O.M.™ Mouse Ig Blocking Reagent effectiveness. If incubation/wash times are lengthened, appropriate negative controls should be run to monitor any decrease in M.O.M.™ Ig Blocking Reagent effectiveness.