USER GUIDE

M.O.M.® (Mouse on Mouse) Immunodetection Kit

Basic

Together we breakthrough™

Cat. No. BMK-2202

Storage Store reagents in original bottles at 2–8 °C. We recommend that the reagents be kept in the box in which they were supplied. Do not freeze.

Description

The Vector® M.O.M. (Mouse on Mouse) Immunodetection Kit is designed specifically to localize mouse primary antibodies on mouse tissues.

The M.O.M. Immunodetection Kit can be used with normal and genetically engineered mouse models, including transgenic, xenograft, knock out and other mutant strains.

Kit Components

Product Name	Volume
M.O.M. Blocking Reagent	1 ml
M.O.M. Protein Concentrate	6 ml
M.O.M. Biotinylated Anti-Mouse IgG	0.1 ml

The 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.

Reagent Not Supplied

The M.O.M. Basic Immunodetection Kit is designed to be used with an avidin- or streptavidin-based enzyme or fluorescence detection system (not included).

Preparation of M.O.M. Working Solutions

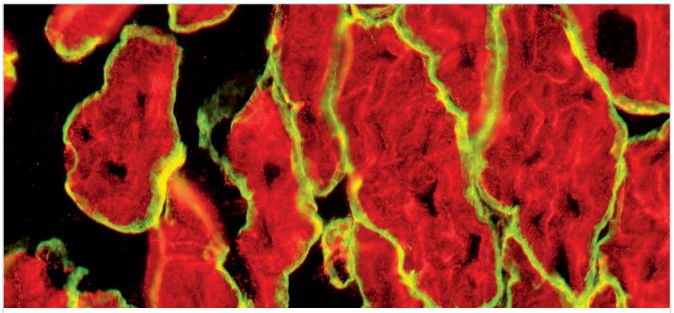
- M.O.M. Mouse IgG Blocking Reagent: add 2 drops (90 µl) of M.O.M. Blocking Reagent stock solution to 2.5 ml of PBS or TBS. †
- M.O.M. Diluent: add 600 µl of M.O.M. Protein Concentrate stock solution to 7.5 ml of PBS or TBS. ††
- M.O.M. Biotinylated Anti-Mouse IgG Reagent: add 10 µl of M.O.M. Biotinylated Anti-Mouse IgG 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, 11.

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. Wash for 5 minutes in tap water.



Skeletal Muscle - Double Label: Muscle-specific actin (m) M.O.M. Basic Immunodetection Kit, Avidin DCS, Texas Red™ (red), Alpha-sarcoglycan (m), M.O.M. Fluorescein Immunodetection Kit (green)

2. If antigen unmasking is required, perform this procedure using a Antigen Unmasking Solution, Citrate-based, pH 6.0 (H-3300) or Tris-based, pH 9.0 (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 Avidin/Biotin Blocking Kit (SP-2001) or Streptavidin/Biotin Blocking Kit (SP-2002).
6. Incubate for 1 hour in working solution of prepared M.O.M. Mouse IgG Blocking Reagent.
7. Wash 2 x 2 minutes in PBS or TBS**.
8. Incubate for 5 minutes in working solution of prepared M.O.M. Diluent**.
9. Dilute primary antibody in M.O.M. Diluent to the appropriate concentration. Tip off excess M.O.M. Diluent from sections and apply diluted primary antibody. Incubate for 30 minutes**.
10. Wash for 2 x 2 minutes in PBS or TBS**.
11. Apply working solution of prepared M.O.M. Biotinylated Anti-Mouse IgG Reagent. Incubate sections for 10 minutes**.
12. Wash for 2 x 2 minutes in PBS or TBS.
13. Apply the appropriate avidin- or streptavidin-based detection system.

* 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.

Detailed product listings, specifications, protocols and additional support information such as our Troubleshooting Guide: Mouse Antibodies on Mouse Tissue are available on our website: vectorlabs.com

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