

Detection of MEK-1 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[Normal Rabbit IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Staining Kit: Vectastain Elite ABC Kit (Rabbit IgG)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-6101

Note: This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Rabbit Anti-MEK-1 Polyclonal Antibody (C-18)

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

www.scbt.com

1-800-457-3801

Catalog # sc-219

Staining Procedure

Positive Control Tissue: Brain
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.
3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

Vectastain Rabbit Elite Staining Kit
Exp. Date _____ New Kit: yes / no

4. Apply the block from the Rabbit Elite Kit, and incubate for 20 minutes at room temperature.

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

5. Avidin / Biotin Blocking Kit

Lot # _____ Exp. Date _____ New Kit: yes / no
Apply avidin block for 15 minutes at room temperature.
Quick rinse in 1X wash buffer.
Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

6. Apply primary antibody at a 1:1000 dilution, and incubate for 1 hour at room temperature.
Lot # _____ Exp. Date _____

For negative control slides, dilute normal rabbit IgG so that its IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:1000 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.

Lot # _____ Exp. Date _____

7. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
8. Apply the secondary antibody from Rabbit Elite Kit, and incubate for 30 minutes at room temperature.
9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Apply the label complex from the Rabbit Elite Kit, and incubate for 30 minutes at room temperature.
(Prepare at least 30 minutes prior to use.)
11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. Apply the DAB chromogen, and incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)
Lot # _____ Exp. Date _____ New Kit: yes / no
13. Rinse the slides in tap water 3 minutes.
14. Counterstain with hematoxylin for 20 seconds.
15. Rinse the slides in tap water until water is clear.
16. Gently agitate slides in 1X wash buffer until they turn blue.
17. Dehydrate through the following solutions:

95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

18. Coverslip

Updated 01/11/07