

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

Reagents

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information

Kit: Rabbit IgG Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6101

Note: The Vector Rabbit Elite Kit contains solutions needed to make the block, secondary and label antibodies.

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

Negative Control Serum: Normal Rabbit Serum
Jackson ImmunoResearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

Staining Procedure

Positive Control Tissue: Normal brain

Stain Localization: Cytoplasmic

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 Automation Buffer	2 times	5 minutes

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

Vector Rabbit Elite Kit: Exp Date _____

3. Apply blocking solution from the Vector Rabbit Elite Kit and incubate for 20 minutes at room temperature.

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

4. Apply the Avidin Biotin Blocking Kit

Lot# _____ Exp Date _____ New Kit: yes / no

Apply avidin block - 15 minutes at room temperature.

Quick rinse in 1X Automation Buffer.

Apply biotin block - 15 minutes at room temperature.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

5. Apply primary antibody (MEK-1) at a 1:1000 dilution and incubate for one hour at room temperature.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of the normal rabbit serum to match the protein concentration of the primary antibody (MEK-1). Use this to make a 1:1000 dilution. Apply to the slides and incubate for one hour at room temperature.

Lot# _____ Reconstituted Date _____

6. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
7. Apply secondary antibody from the Rabbit Elite Kit and incubate for 30 minutes at room temperature.
8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
9. Apply label antibody from the Rabbit Elite Kit and incubate for 30 minutes at room temperature.
10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
11. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot#_____ Exp Date_____ New Kit yes / no
12. Rinse in tap water 3 minutes.
13. Counterstain with Modified Harris Hematoxylin for 20 seconds.
14. Rinse in tap water until water is clear.
15. Gently agitate slides in 1X Automation buffer until they turn blue.
16. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

17. Coverslip

Updated 01/11/07