

Detection of EphA3 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 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 and secondary reagents.

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary antibody: Rabbit anti-EphA3 (C-19) Polyclonal Antibody
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-919

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

Label antibody: Peroxidase-conjugated Streptavidin SS Label

Biogenex

San Ramon, CA 94583

www.biogenex.com

1-800-421-4149

Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Mouse Brain

Stain Localization: Cytoplasmic (purkinjes of cerebellum)

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.
3. Unmasking Technique using the decloaker.
Add 500ml distilled water to the pan of the decloaker.
Place a full rack of slides in a Tissue Tek™ container containing 250ml of 1X citrate buffer solution.
Decloak for 5 minutes. Pressure _____
Depressurize for 10 minutes.
Remove pan top and cool for 10 minutes. Temperature before cooling _____
Rinse in distilled water two times for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Apply blocking solution from the Vector Rabbit Elite kit and incubate for 20 minutes at room temperature.
Exp. Date _____ New Kit: yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

6. Apply Avidin/Biotin block

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

Apply avidin block - 15 min at room temperature.

Quick rinse in 1X Automation Buffer

Apply biotin block - 15 min at room temperature.

Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (EphA3) at a 1:400 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 the protein concentration of the primary antibody (EphA3), and use this to make a 1:400 dilution. Apply to the slides and incubate for one hour at room temperature.

Lot# _____ Reconstitution Date _____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary 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 Biogenex SuperSensitive Label antibody and incubate for 30 minutes at room temperature.

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. 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

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until blue.

18. Dehydrate through the following solutions.

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

19. Coverslip

Updated 06/29/06