

Identification of Neomycin in Formalin-Fixed Paraffin Embedded Mouse Tissue

Reagents:

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

Antibody Information

Dako Protein Block Serum-Free--Ready to use
Carpinteria CA
www.dakousa.com
1-800-235-5763
Catalog # X0909

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

Primary Antibody: Rabbit anti-Neomycin
Upstate Cell Signaling Solutions.
Lake Placid, NY 12945
Catalog #06-747
Suggested dilution: 1:300
www.upstate.com
1-800-233-3991

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

DAKO LSAB+ System HRP

DAKO Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Code No. K0690

Staining Procedure

-Positive Control Tissue: Tissue with neomycin insert

-Stain localization: Cytoplasm

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 changes	5 minutes
100% ETOH	2 changes	3 minutes
95% ETOH	2 changes	3 minutes
Automation Buffer	2 changes	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 Techniques using the decloaker.

Add 500 ml D/W to the pan of the decloaker.

Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.

Decloak for 5 minutes. Pressure _____

Depressurize for 10 minutes.

Remove pan top and cool for 10 min. Temp _____

Rinse in D/W, 2x for 3 min each

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

5. Use Dako Protein Block serum-free for 10 minutes at room temperature.

Lot# _____ Exp Date _____

DO NOT RINSE SLIDES.

6. Apply Avidin/Biotin block

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

Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.

No wash, wipe excess block and apply primary antibody

7. Apply primary antibody (Rabbit anti-Neomycin) at 1:300 dilutions and incubate for one hour.

Lot # _____ Exp date _____

For negative control slides, normalize the protein concentration of the normal rabbit serum with the protein concentration of the primary antibody (Rabbit anti-Neomycin) and use this to make the 1:300 dilution. Apply to slides and incubate for one hour.

Lot# _____ Reconstituted Date _____

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

9. Apply Link(yellow bottle) from LSAB+ Kit for 20 minutes.

LSAB Kit Lot # _____ Exp Date _____

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

11. Apply label (red bottle) from LSAB+ kit and incubate for 20 minutes

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. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% ETOH	1 changes	3 minutes
100% ETOH	3 changes	3 minutes
xylene	2 changes	5 minutes

18. Coverslip
updated 2/9/04