Identification of Neurofilament in Formalin-Fixed, Paraffin-Embedded Rodent Tissue

Reagents:

1X Automation Buffer

3% Hydrogen Peroxide

Antibody Diluent

Citrate Buffer

DAB Chromagen

Hematoxylin

Antibody Information

Dako LSAB2 System HRP
Dako Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Code No. K0609
*This kit includes reagents needed for link and label antibodies.

Primary antibody: Mouse anti-Neurofilament Protein
Dako Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Code No. M0762

Negative control serum: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Staining Procedure

-Positive Control Tissue: brain
-Stain Localization: cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	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 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

NO BLOCK REQUIRED

4. Apply primary antibody (Neurofilament) at 1:100 dilution and incubate for 10 mins.
Lot#_____Exp Date_____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (Neurofilament) and use this to make the 1:100 dilution for 10 min.
Lot#_____ Reconstituted Date_____

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

LSAB2 Kit Lot#_____ Exp Date_____

6. Apply Link antibody (Yellow bottle) from LSAB2 kit for 10 minutes.

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

8. Apply label antibody (Red bottle) from LSAB2 kit for 10 minutes

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

10. 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

11. Rinse in tap water 3 minutes.

12. Counterstain with Modified Harris Hematoxylin for 30 seconds.

13. Rinse in tap water until water is clear.

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

15. Dehydrate through the following solutions.

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

16. Coverslip.
updated 07/29/04