Detection of Neurofilament Protein (NFP) in Formalin-Fixed, Paraffin-Embedded Mouse and Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
DAB Chromagen
Hematoxylin

Primary Antibody: Mouse Anti-Neurofilament Protein Antibody
Dakocytomation 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 Kit: LSAB 2 System-HRP
Dakocytomation Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Code No. K0609

Note: This kit includes reagents needed for the secondary antibody (link) and label complex.

Staining Procedure

Positive Control Tissue: Brain
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
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.

4. Heat-Induced Epitope Retrieval Using The Decloaker
   Add 500 ml of distilled water to the pan inside the decloaker.
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides*_____
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

6. Apply the primary antibody at a 1:100 dilution, and incubate for 10 minutes at room temperature.
   Lot #_____Exp Date_____

   For negative control slides, dilute the protein concentration of the normal mouse serum to match that of the primary antibody. Make a 1:100 dilution from this normalized serum, and apply to the slides. Incubate for 10 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

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

   LSAB 2 Kit
   Lot # _____ Exp Date_____

8. Apply the Link (yellow bottle) from the LSAB 2 Kit, and incubate for 10 minutes at room temperature.

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

10. Apply the Label (red bottle) from the LSAB 2 Kit, and incubate for 10 minutes at room temperature.

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each

12. Apply the DAB chromagen, 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 Harris 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:

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

18. Coverslip

Updated 07/29/04