Identification of IL1 alpha in PLP Fixed Mouse Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Antigen Retrieval Solution
Hematoxylin
PLP fixative

Antibody Information

Primary antibody: Biotin-conjugated Rabbit anti-IL1 alpha
Antigenix America
Huntington Sta, NY 11746
1-800-558-1008
Catalog # RMF326B

Negative Serum Control: Biotin conjugated normal rabbit serum
Vector Laboratories
30 Ingold Rd
Burlingame CA 94010
1-800-227-6666
Catalog # BI-1005

Label: Biogenex supersensitive label
Biogenex
San Ramon CA 94583
Catalog # HK-330-9K

Comment: the following protocol with the listed antibody works best in tissues fixed overnight in PLP (periodate-lysine-paraformaldehyde) fixative.
Bouin's-fixed tissue are applicable for this procedure. Formalin and zinc-formalin fixation is not acceptable for this commercial antibody.

Staining Procedure

-Positive Control Tissue: PLP fixed tissues

-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: Steamer
Place slides in 1X Citrate Buffer and steam for 35 minutes.
Remove slides from steamer and cool for 20 minutes.Temp_____
Stop reaction by rinsing slides in D/W.
Place slides in 1X Automation buffer for 5 minutes.

4. Apply primary antibody (Rabbit anti-IL alpha) at a 1:30 dilution and incubate for 1 hr at room temperature.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of biotin-conjugated normal rabbit serum to the protein concentration of the primary antibody.
Lot#_____ Reconstituted Date_____
(note: if you are reconstituting a new bottle of the serum DO NOT VORTEX)

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

6. Apply Biogenex super sensitive label and incubate for 30 minutes.
Lot#_____ Exp. Date_____

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

8. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
Lot#_____ Exp. Date_____ New Kit yes / no
(Add 1 drop of DAB per ml of substrate)

9. Rinse in tap water 3 minutes.

10. Counterstain with Modified Harris Hematoxylin for 1 min.

11. Rinse in tap water until water is clear.

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

13. Dehydrate through the following solutions.

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

15. Coverslip

updated 6/25/2003