

## 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.

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### 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

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