Detection of iNOS in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information:

Kit: Vector Elite Rabbit IgG ABC kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK 6101.
*The Vector Rabbit Elite Kit contains solutions needed to make the secondary and label antibodies

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

Primary antibody: i-NOS Polyclonal Antibody
Transduction Laboratories
Lexington, KY 40511
1-800—227-4063
Catalog # N32030

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

Staining Procedure

-Positive Control Tissue: Inflammatory cells in LPS treated liver.
LPS (Lipopolysaccharide) induces iNOS.

-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. Perform Heat Induced Epitope Retrieval using steamer
Place a full rack of slides in 250mls of citrate buffer.
Heat in steamer for 30 minutes.
Remove and cool slides in citrate buffer for 10 minutes. Temp. _____
Rinse slides 3 X 2 minutes in distilled water
Place in 1X Automation buffer for 5 minutes.

4. Apply block from Vector Rabbit Elite kit and incubate for 20 minutes at room temperature.
Exp Date_____ New Kit: yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

5. 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.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

6. Apply primary antibody (iNOS) at a 1:25 dilution and incubate for one hour at room temperature.
Lot#_____ Aliquoted yes / no Date Aliquoted _____

For negative control slides, normalize the protein concentration of normal rabbit serum to the protein concentration of the primary antibody (iNOS) and use this to make the 1:25 dilution. Apply normal rabbit serum to the slides and incubate for one hour at room temperature.
Lot#_____ Reconstituted Date_____

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

8. Apply secondary antibody from Vector Elite kit and incubate for 30 minutes at room temperature.

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

10. Apply Label antibody and incubate for 30 minutes at room temperature. (Prepare 30 minutes prior to use.)

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

12. 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

13. Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

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

17. Dehydrate through the following solutions.

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

18. Coverslip

updated 01/20/05