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

Reagents:

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

Antibody Information

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-001

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

Primary antibody: Rabbit anti-eNOS (NOS3)
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog# sc-654

Negative Serum Control: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog# 011-000-001

Secondary antibody: Biotinylated goat anti-rabbit IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog# BA-1000

Label antibody: Vector Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog# PK-6101

Staining Procedure

-Positive Control Tissue: Heart endothelial cells
-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

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

5. Block with 10% Normal Goat Serum and incubate for 20 minutes.
Lot#_____ Reconstituted Date_____

DO NOT RINSE SECTIONS WITH BUFFER

6. 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.
Wipe excess block.

DO NOT RINSE SECTION WITH BUFFER

7. Apply primary antibody (Rabbit anti- eNOS) at 1:600 dilution and incubate for one hour at room temperature.
Lot#_____ Exp Date_____

Normalize the protein concentration of the normal rabbit serum with the protein concentration of the primary antibody (eNOS) and use this to make the 1:600 dilutions. Apply normal rabbit serum to the slides and incubate for one hour at room temperature.
Lot#_____ Reconstituted Date_____

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

9. Apply secondary (biotinylated goat anti-rabbit) at 1:800 and incubate for 30 minutes.
Lot#_____ Reconstituted Date_____

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

11. Apply Vector Elite Label and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)
Exp. Date _____ New Kit: yes / no

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

13. 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

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

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

18. Dehydrate through the following solutions.

95% Ethanol	1 times	3 minutes
100% EtOH	3 times	3 minutes
Xylene	2 times	5 minutes

19. Coverslip.
updated 12/29/04