

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

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

Citrate Buffer

[DAB Chromagen](#)

Antigen Retrieval Solution

Hematoxylin

Antibody Information

Rabbit Elite Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #PK-6101

*This contains everything need to make blocking reagent, secondary antibody and label antibody.

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody: Rabbit anti-Phospho-eNOS3 (Ser1177)

Cell Signaling Technology

Beverly, MA 01915

877-678-CELL

Catalog #9571L

Suggested dilution: 1:10

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

Staining Procedure

-Positive Control Tissue: not one for this experiment

-Stain Localization: Cytoplasmic

Cut 2 sequential 6 micron sections onto poly-l-lysine coated slides. Do not pre-heat treat the slides in histology. The slides should be allowed to air dry.

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.

3. Apply blocking reagent from Rabbit Elite kit and incubate for 20 minutes.

Exp. Date _____ New kit yes / no

Wipe excess reagent from around tissue section. DO NOT RINSE SECTIONS WITH BUFFER.

4. Apply Avidin/Biotin block

New kit yes / no Lot# _____

Apply avidin block - 15 min @ RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min @ RT.

No wash, wipe excess block and apply primary antibody

5. Apply primary antibody (Rabbit anti-Phospho-eNOS) at a 1:10 dilution and incubate for one hour. (Concentration @ 40 ug/ml)

Lot# _____ Aliquoted yes / no Date Aliquoted _____

1:10

Normalize the protein concentration of the Normal Rabbit Serum with the protein concentration of the primary antibody (Phospho-eNOS) and use this to make the following dilutions:

Lot# _____ Reconstituted Date _____

1:10

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

7. Apply secondary antibody from Rabbit Elite kit and incubate for 30 minutes.

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

Apply Vector Elite Label and incubate for 30 minutes. (Prepare at least 30 mins prior to use) 2 drops Reagent A + 5 ml diluent -> Mix and then add 2 drops Reagent B

Exp. Date _____ New kit yes / no

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

10. 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

11. Rinse in tap water 3 minutes.

12. Counterstain with Modified Harris Hematoxylin for 30 seconds.

13. Rinse in tap water until water is clear.

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

15. Dehydrate through the following solutions.

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

19. Coverslip.