

Detection of Cox2 in Formalin-Fixed, Paraffin-Embedded Mouse 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-mouse Cox2

Cayman Chemical

Ann Arbor, MI 48108

www.caymanchem.com

1-800-364-9897

Catalog #160106

Negative control serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Concentration: 60 mg/ml

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 Standard Elite Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6100

Staining Procedure:

Positive Control Tissue: Highest level of expression located at the distal vas deferens where it inserts into the bladder. Weak staining at the proximal end of the vas defereas.
Stain localization: Peri-Nuclear/Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	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 microwave oven.
Place a full rack of slides in tissue tek container containing 200ml of distilled water.
Microwave for 5 minutes at level 5
Cool for 1 minute (Add more distilled water if necessary)
Microwave for 5 minutes at level 5. Temp after Microwaving _____
Cool 20 minutes at room temperature.
Rinse in distilled water two times for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block with 5% Normal Goat Serum for 20 minutes at room temperature.
Lot# _____ Reconstituted Date _____

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

6. Apply Avidin/Biotin block
Lot# _____ Exp. Date _____ New Kit: yes / no
Apply avidin block - 15 min at RT.
Quick rinse in 1X Automation Buffer.
Apply biotin block - 15 min at RT.
No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Cox2) at 1:4000 dilution and incubate for one hour at room temperature.
Lot# _____ Aliquoted yes / no Date Aliquoted _____

For the negative control slides, match the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Cox2), and use this to make the 1:4000 dilution. Apply 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 antibody (Biotinylated goat anti-rabbit) at 1:500 dilution and incubate for 30 minutes at room temperature.
Lot# _____ Reconstituted Date _____

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

11. Apply Label antibody and incubate for 30 minutes at room temperature.
(Prepare 30 minutes 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 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until blue.

18. Dehydrate through the following solutions.

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

19. Coverslip

Updated 04/06/06