

Identification of PKC δ in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information:

Dako Protein Block Serum-Free

Dako Corporation

Carpinteria CA 93013

www.dakousa.com

1-800-235-5763

Catalog# X0909

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody: Mouse anti-PKC δ

BD Pharmingen

Distributed by Transduction Labs

Lexington, KY 40511

www.bdpharma.com

1-800-227-4063

Catalog# 610090

Negative control serum: Normal Mouse Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog# 015-000-001

Kit: Dako LSAB+ System HRP

DakoCorporation

Carpinteria CA 93013

www.dakousa.com

1-800-235-5763

Catalog #K06901

Staining Procedure

-Positive Control Tissue: mouse spleen

-Stain Localization: Cytoplasmic / Membrane

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. Apply Dako Serum-free Protein Block and incubate for 10 minutes at room temperature.

Lot# _____ Exp 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 AB.

Apply biotin block - 15 min at RT.

Wipe excess block.

DO NOT RINSE SLIDES. CONTINUE TO PRIMARY ANTIBODY.

7. Apply primary antibody (PKCØ) at a 1:10 dilution and incubate for one hour at room temperature.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (PKCØ) and use this to make the 1:10 dilution. Apply normal mouse 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 Link antibody (Yellow bottle) from LSAB+ kit and incubate for 30 minutes at room temperature.

Kit Lot# _____ Exp Date _____

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

11. Apply label antibody (Red bottle) from LSAB+ kit and incubate for 30 minutes at room temperature.

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 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

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

19. Coverslip.
updated 07/07/05