Identification of Calretinin in Formalin-Fixed, Paraffin Embedded Rodent Tissue

Reagents:

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

Antibody Information

Block: Dako Protein Block Serum-Free
DAKO Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Code no. X0909

Dako LSAB+ System HRP
Dako Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Code No. K06901
*This kit includes reagents needed for link 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: Goat anti-Calretinin
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Cat# sc-11644

Negative Serum Control: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-121

Staining Procedure

-Positive Control Tissue: mouse brain

-Stain localization: cytoplasmic, in the neurons.

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 Protein block for 10 mins.
Lot#_____ Exp Date_____

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

6. Apply the Avidin Biotin Blocking kit

Lot#_____ Exp Date_____ New Kit:: yes / no
Apply avidin block - 15 min at RT.
Quick rinse in 1X Automation Buffer. (Do not leave slides sitting in buffer)
Apply biotin block - 15 min at RT.
Wipe excess block.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody at 1 :500 dilution and incubate for 30 min at room temperature.
Lot #_____ Aliquoted yes / no Date Aliquoted_____

For negative control slides, normalize the protein concentration of normal goat serum to the protein concentration of the primary antibody and use this to make 1:500 dilution. Apply to slides and incubate for 30min at room temperature.
Lot # _____ Reconstituted Date_____

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

9. Apply Link (Yellow Bottle) from LSAB kit for 30 minutes.
LSAB Kit lot #_____ Exp Date_____

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

11. Apply Label (Red Bottle) from LSAB kit and incubate for 30 minutes.

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 clear.

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

18. Dehydrate through the following solutions.

95% ETOH	1 changes	3 minutes
100% ETOH	3 changes	3 minutes
xylene	2 changes	5 minutes

19. Coverslip
update 05/17/04