Detection of Calretinin in Formalin-Fixed, Paraffin-Embedded Mouse and Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
DAB Chromagen
Hematoxylin

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use
Dakocytomation Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Code No. X0909

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 Polyclonal Antibody (N-18)
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-11644

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

Staining Kit: LSAB+ System-HRP
Dakocytomation Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Code No. K0690

Note: This kit includes reagents needed for the secondary antibody (link) and label complex.

Staining Procedure

Positive Control Tissue: Brain
Stain Localization: Cytoplasm in the neurons

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker
   Add 500 ml of distilled water to the pan inside the decloaker.
   Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
   (Insert blank slides into any empty slots in the rack to ensure even heating of slides)
   Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides*_____
   Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

6. Block with the Dako Protein Blocking Reagent for 10 minutes at room temperature.
   Lot #_____ Exp Date_____

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

7. Avidin / Biotin Blocking Kit
   Lot #_____ Exp Date_____ New Kit: yes / no
   Apply avidin block for 15 minutes at room temperature.
   Quick rinse in 1X Wash Buffer.
   Apply biotin block for 15 minutes at room temperature.

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BUFFER.

8. Apply the primary antibody at a 1:500 dilution, and incubate for 30 minutes at room temperature.
   Lot #_____ Date Aliquoted_____

   For negative control slides, dilute the protein concentration of the normal goat serum to match that of

the primary antibody. Make a 1:100 dilution from this normalized serum, and apply to the slides. Incubate for 30 minutes at room temperature.
Lot #_____ Date Reconstituted_____

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

LSAB+ Kit
Lot # _____ Exp Date_____

10. Apply the Link (yellow bottle) from the LSAB+ Kit, and incubate for 30 minutes at room temperature.

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

12. Apply the Label (red bottle) from the LSAB+ Kit, and incubate for 30 minutes at room temperature.

13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each

14. Apply the DAB chromagen, and incubate in the dark for 6 minutes at room temperature.
(Add 1 drop of DAB per ml of substrate)
Lot #_____ Exp Date_____ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until they turn blue.

19. Dehydrate through the following solutions:

Solution	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

Updated 05/17/04