Detection of Calbindin D-28K in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
1X Citrate Buffer
Normal Rabbit IgG – Affinity Purified
DAB Chromogen
Hematoxylin

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use
Dakocytomation Corporation
Carpinteria, CA 93013
www.dako.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: Rabbit Anti-Calbindin D-28K Polyclonal Antibody
Millipore
Billerica, Massachusetts 01821
www.millipore.com
1-800-645-5476
Catalog # AB1778

Staining Kit: LSAB+ System-HRP
Dakocytomation Corporation
Carpinteria, CA 93013
www.dako.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: Cytoplasmic

1. 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 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 BLOCK.

8. Apply the primary antibody at a 1:750 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

   For negative control slides, dilute normal rabbit IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:750 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

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. 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. 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 chromogen. 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 hematoxylin for 20 seconds.

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

18. Gently agitate slides in 1X wash buffer until the tissues turn blue.

19. Dehydrate through the following solutions:

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

20. Coverslip

Updated 06/09/07