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**
**Normal Goat 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: 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

**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 – Neurons  
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Repetitions</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylene</td>
<td>2 times</td>
<td>5 minutes</td>
</tr>
<tr>
<td>100% Ethanol</td>
<td>2 times</td>
<td>3 minutes</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>2 times</td>
<td>3 minutes</td>
</tr>
<tr>
<td>1X Wash Buffer</td>
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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 1:500 dilution. Incubate for 30 minutes at room temperature.  
   **Lot #______________ Exp. Date ______________**

For negative control slides, dilute normal goat IgG so that it’s IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:500 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 30 minutes 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:

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20. Coverslip

*Updated 05/17/04*