Detection of CD34 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

**1X Wash Buffer**
**3% Hydrogen Peroxide**
**1% BSA Diluent**
**1X Citrate Buffer**
**DAB Chromogen**
**Hematoxylin**
**1% Dry Milk**

**Blocking Serum: Normal Rabbit Serum**
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

**Avidin / Biotin Blocking Kit**
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

**Primary Antibody: Rat Monoclonal Antibody To CD34**
Abcam Inc
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog # ab8158

**Negative Control Serum: Purified Rat IgG2a, κ Isotype Control Antibody**
BioLegend
San Diego, CA 92121
www.biolegend.com
1-877-246-5343
Catalog # 400502

**Secondary Antibody: Biotinylated Rabbit Anti-Rat IgG (H+L)**
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-4001
Staining Procedure

Positive Control Tissue: Embryos (stem cells), glomeruli of kidney, and lung capillaries: endothelial cells and hematopoietic cells

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>
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<td>2 times</td>
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</tr>
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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.

   The diluent for the block, primary antibody, negative control reagent, and secondary antibody will consist of a 1:1 mixture of 1% BSA diluent and 1% milk. The 1% milk should be prepared in distilled water.

6. Block with 10% normal rabbit serum for 20 minutes at room temperature.
   Lot #_________________ Date Reconstituted_________________

   DO NOT RINSE 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 primary antibody at a 1:800 dilution. Incubate for 1 hour at room temperature.
   Lot #______________ Exp. Date ________________

For negative control slides, dilute rat IgG2a control serum so that it’s IgG2a protein concentration matches that of the primary antibody (if necessary). Then make a 1:800 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.

10. Apply the rabbit anti-rat secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
    Lot #______________ Date Reconstituted______________

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

12. Apply the Streptavidin SS Label. Incubate for 30 minutes at room temperature.
    Lot #______________ Exp. Date ________________

13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

14. Apply the DAB chromogen 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 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