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 Chromagen
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 # ab8158-100

Negative Control Serum: Normal Rat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 012-000-001

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

Label Complex: Peroxidase-Conjugated Streptavidin SS Label
Biogenex Laboratories
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog # HK330-9K

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:

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.

   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 BUFFER.

8. Apply primary antibody at a 1:100 dilution, and incubate for 1 hour at room temperature.
   Lot #_____ Date Aliquoted_____

For negative control slides, dilute the protein concentration of the normal rat serum to match that of the primary antibody. Make a 1:100 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.
Lot #_____ Date Reconstituted_____

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

10. Apply the rabbitt anti-rat secondary antibody at a 1:500 dilution, and 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, and 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 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 12/19/06