

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

## Reagents

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

1% Dry Milk prepare in distilled water.

## Antibody Information

Blocking serum: Normal Rabbit Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 011-000-001

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # SP-2001

Primary antibody: Rat Monoclonal to CD34 Antibody

Abcam Inc

Cambridge, MA 02139

[www.abcam.com](http://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](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 012-000-001

Secondary antibody: Biotinylated Rabbit anti-rat IgG

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # BA-4001

Label antibody: Peroxidase-conjugated Streptavidin SS Label

Biogenex

San Ramon, CA 94583

[www.biogenex.com](http://www.biogenex.com)

1-800-421-4149

Catalog # HK330-9K

**Staining Procedure**

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

Stain Localization: Endothelial cells

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 Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.
2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
3. Unmasking technique using the decloaker.  
Add 500 ml distilled water to the pan of the decloaker.  
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.  
Decloak for 5 minutes. Pressure \_\_\_\_\_  
Depressurize for 10 minutes.  
Remove pan top and cool for 10 minutes. Temperature before cooling \_\_\_\_\_  
Rinse in distilled water twice for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

**The diluent for the block, primary antibody, negative antibody, and secondary antibody will consist of a 1:1 mixture of 1% BSA diluent and 1% milk.**

5. Block with 10% Normal Rabbit Serum for 20 minutes at room temperature.  
Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

6. Apply the Avidin Biotin Blocking Kit  
Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_ New Kit: yes / no  
Apply avidin block - 15 minutes at room temperature.  
Quick rinse in 1X Automation Buffer.  
Apply biotin block - 15 minutes at room temperature.  
No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (CD34) at a 1:100 dilution and incubate for one hour at room temperature.  
Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, normalize the protein concentration of the normal rat serum to match the protein concentration of the primary antibody (CD34) and use this to make a 1:100 dilution. Apply to slides and incubate for one hour at room temperature.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply rabbit anti-rat secondary antibody at a 1:500 dilution and incubate for 30 minutes at room temperature.  
Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Biogenex SS Label and incubate for 30 minutes at room temperature.  
Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.  
(Add 1 drop of DAB per ml of substrate)  
Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation buffer until they turn blue.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

*Updated 12/19/06*