

# 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,  $\kappa$  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

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 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:

Solutions	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