

Detection of Pecam (CD31) in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[EDTA](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information:

Blocking Serum: Normal Donkey Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog# 017-000-001

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody : Goat anti-Pecam-1 (CD31)

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

www.scbt.com

1-800-457-3801

Catalog# sc-1506

Negative control serum: Normal Goat Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #005-000-001

Secondary antibody: Biotinylated donkey anti-goat IgG
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 705-065-147

Label antibody: StriAviGen Super Sensitive Predilute Label Antibody
Biogenex Laboratories
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog #HK330-5K

Staining Procedure

- Positive Control Tissue: Blood vessels: endothelial cells
- Stain Localization: cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	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 Techniques using the decloaker.
Add 500 ml D/W to the pan of the decloaker.
Place full rack of slides in 200 ml of EDTA (1:5) and place in the decloaker
Decloak for 5 minutes. Pressure _____
Depressurize for 10 minutes.
Remove pan top and cool for 10 min. Temp _____
Rinse in D/W, 2x for 3 min each
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block in 5% Normal Donkey Serum for 20 minutes.

Lot# _____ Reconstituted Date _____

6. Apply Avidin/Biotin block

Lot# _____ Exp Date _____ New Kit: yes / no

Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.

Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Pecam-1) at 1:500 dilution and incubate for one hour.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of the normal goat serum to the protein concentration of the primary antibody (Pecam-1) and use this to make the 1:500 dilution and incubate for one hour.

Lot# _____ Reconstituted Date _____

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

9. Apply secondary antibody (Biotinylated donkey anti-goat) at a 1:300 dilution and incubate for 30 minutes.

Lot# _____ Reconstituted Date _____

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

11. Apply Label (Biogenex) antibody and incubate for 30 minutes.

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 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

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

19. Coverslip
updated 12/27/04