

Detection of Von Willebrand Factor (Factor 8) in Formalin Fixed, Paraffin-Embedded Rodent Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Carenzyme II \(Pepsin\)](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information:

Kit: Rabbit Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com

1-800-227-6666

Catalog: PK-6101

*The Vector Rabbit Elite Kit contains solutions needed to make the secondary and label antibodies.

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Retrieval Reagent: Carenzyme II (Pepsin)

Biocare Medical
Walnut Creek CA
www.biocare.net

1-800-799-9499

Catalog# pep956H

Primary antibody: Rabbit Anti-Human Von Willebrand Factor (Factor 8)

Biocare Medical

1-800-799-9499

Concentration: 1 mg/ml

Catalog #CP039A

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

Staining Procedure

- Positive Control Tissue: Endothelial cells
- Stain Localization: Cytoplasmic

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 one change of 1X Automation Buffer for 5 minutes at room temperature.
3. Place slides in one change of 1X Automation Buffer for 5 minutes at 37 degrees.
4. Place slides on rack at 37 degrees.

Apply ready-to-use Carezyme-II solution to slides for 5 minutes. (Carezyme II should be at RT prior to use)

Comment: Heat-induced epitope retrieval does not work well with this antibody. Enzyme treatment is the preferred method of antigen retrieval.

5. Rinse slides in distilled water for 1 minute.
6. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes.
7. Apply blocking solution from the Vector Rabbit Elite kit and incubate for 20 minutes at room temperature.

Exp. Date _____ New Kit: yes / no

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

8. Apply the Avidin Biotin Blocking kit

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

Apply avidin block - 15 min at RT.

Quick rinse in 1X Automation Buffer.

Apply biotin block - 15 min at RT.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

9. Apply primary antibody (Rabbit anti-Human Factor 8) at a 1:500 dilution and incubate one hour at room temperature.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Factor8) and use this to make a 1:500 dilution. Apply and incubate for one hour at room temperature.

Lot# _____ Reconstituted Date _____

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

11. Apply secondary antibody from the Rabbit Elite Kit and incubate for 30 minutes at room temperature.

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

13. Apply Label antibody from Vector Rabbit Elite Kit and incubate for 30 minutes at room temperature. (Prepare at least 30 mins prior to use)

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

15. 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

16. Rinse in tap water 3 minutes.

17. Counterstain with Modified Harris Hematoxylin for 30 seconds.

18. Rinse in tap water until clear.

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

20. Dehydrate through the following solutions.

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

21. Coverslip

Updated 04/13/06