



Immunohistochemical Staining for von-Willebrand Factor (vWF) Using Affinity Purified Goat antibody and Vectastain-Elite ABC Kit

Introduction

von Willebrand Factor (vWF) is an adhesive protein that circulates in plasma at about 10 ug/ml and is also found in platelets and endothelial cells. vWF is found as multimers of disulphide-linked 220,000 Dalton subunits and its molecular weight ranges from 0.5-20 million Daltons. vWF is involved in the transport of Factor VIII (antihemophilic factor) and plays an important role in platelet adhesion and aggregation. von Willebrand Disease (vWD) can be due to a quantitative deficiency of vWF (vWD Types I & III) or to qualitative disorders resulting from the production of functionally abnormal protein (vWD Type II). The procedure outlined here was developed for immunohistochemical staining of vWF in endothelial cells at the light microscopy level and uses Product # GAVWF-AP as the primary antibody, Biotinylated rabbit anti-goat IgG (Product # BA-5000, Vector Laboratories, Burlingame, CA) as the second antibody. Detection of biotinylated antibody was performed using the VectaStain-Elite detection kit from Vector Labs.

Method

1. Paraformaldehyde fixed tissues samples were embedded in paraffin.
2. Remove paraffin in toluene (2 X 20 min).
3. Put through graded alcohols (100% - 25% (w/v), 5 min each), then rinse in water.
4. Block with methanol-peroxide (0.3% H₂O₂ in MeOH) for 30 minutes.
5. Sections were washed in PBS (10 mM NaHPO₄, pH 7.4, 0.15 M NaCl) for 20 min.
6. Layer on PBS containing a 1/100 dilution of normal rabbit serum for 20 min.
7. Rinse and layer on the primary antibody diluted in PBS containing 1% (w/v) Bovine Serum Albumin and place in a humidity chamber overnight at 4°C.
8. **Note:** For vWF staining we recommend affinity-purified goat anti-vWF (GAVWF-AP) diluted to 0.5 ug/ml. In general, affinity-purified polyclonal antibodies or purified monoclonal antibodies may be used in a concentration range of 0.5 to 5 ug/ml, while whole IgG antibodies or ascites may be used in a concentration range of 5 to 25 ug/ml. Optimal concentrations of each different primary and secondary antibody should always be determined empirically by titration.
9. Wash in PBS for 10 minutes then layer on secondary antibody (biotinylated Rabbit anti-Goat IgG) diluted approximately 1/100 in PBS for 30 minutes at ambient temperature.
10. Wash in PBS for 10 minutes, then apply the VectaStain ABC reagent mixture as per the manufacturer's instructions.
11. Develop in 0.05% (w/v) DAB in 0.05 M Tris-HCl, pH 7.6, containing 0.075% H₂O₂ for 15 minutes at ambient temperature.
12. Rinse in cold water, then counterstain in hematoxylin for 3-5 minutes.
13. Rinse in water, then graded alcohols (25% - 100% (w/v), 5 minutes each).
14. Rinse in 3 changes of toluene then coverslip with permount.