Detection of Rabbit anti-VEGF in paraffin-embedded rat tissue

Antibody Information:
Vector Goat Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #

Contains everything needed to make blocking reagent, secondary antibody and label antibody

Avidin/Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #PK-6105

Primary antibody: (VegF (A-20-G), goat polyclonal)
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog #sc-152-G
Concentration: 200ug/ml

Microwave Information
Panasonic High Power 1200W
Model number NN-S732WL
Conditions for retrieval dependent upon this microwave.

Staining Procedure
-Positive Control Tissue: Islets of Langerhans (pancreas)
-Stain localization: Cell Membrane

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. Perform Heat Induced Epitope Retrieval using MWO

Place a full rack of slides in Tissue Tekô container containing 200 mls 1X citrate buffer.

MWO for 5 minutes at level 3
Cool for 1 minute (Add 50 mls citrate buffer to container)
MWO for 5 minutes at level 3 Temp._____
Cool 20 minutes at room temperature
Rinse in distilled water 3 X 2 minutes each
Place slides in buffer for 5 minutes

4. Apply block from Vector Goat Elite kit and incubate for 20 minutes.

Lot#_____ Exp. Date_____

5. Apply Avidin/Biotin block

Lot#_____ Exp_____New Kit yes / no

Apply avidin block - 15 min @ RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min @ RT.
No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

6. Apply primary antibody VEGF at the following dilutions and incubate for 1 hour at RT.

Lot#_____ Exp Date_____

1:1000

For negative control slides, normalize the protein concentration of normal goat serum to the protein concentration of the primary antibody (VEGF) and make the following dilutions. Apply normal goat serum to the slides and incubate for one hour.

Lot#_____ Reconstituted Date _____

1:1000

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

8. Apply secondary from Vector Goat Elite kit and incubate for 30 minutes.

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

10. (Prepare Label antibody 30 minutes prior to use)
Apply Label antibody from Vector Goat Elite Kit for 30 minutes. 2 drops Reagent A + 5 ml diluent -> Mix and then add 2 drops Reagent B

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

12. 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

13. Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 20 seconds.

15. Rinse in tap water until water is clear.

16. Place slides in 1X AB for 1 minute to ìblueî.

17. Dehydrate through the following solutions.

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

18. Coverslip

updated 8/8/2003