Detection of Vitamin D Receptor in Formalin-Fixed, Paraffin-Embedded Rodent Tissue

Reagents:
1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information:

Block: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #008-000-001

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

Primary antibody : Monoclonal Rat Anti-Vitamin D Receptor
Biomeda Corp.
Foster City, CA 94404
(800)341-8787
Catalog K130
Suggested dilution: 1:10

Negative control: Normal Rat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #012-000-001

Kit used: Vector Rat Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #PK-6104
*This kit contains reagents necessary to make secondary and label antibodies.

Detection of Vitamin D in Paraffin-embedded Rodent Tissue

Staining Procedure
-Positive Control Tissue: Small Intestine
-Stain localization: Nuclear

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 1X citrate buffer 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. Block using 5% Normal Horse Serum for 20 minutes.
Lot#_____ Reconstituted Date_____

5. 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

Apply primary antibody (Monoclonal rat-anti Vitamin D) at the 1:10 dilution and incubate overnight at 4 degrees.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of the normal rat serum to the protein concentration of the primary antibody (Vitamin D) and use this to make the 1:10 dilution and incubate for overnight at 4 degrees.
Lot#_____ Reconstituted Date_____

******* Next Day**********

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

7. Apply the secondary antibody at a 1:500 dilution and incubate for 30 minutes.
Exp Date_____New Kit: yes / no

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

9. Apply the label antibody and incubate for 30 minutes (Prepare atleast 30 min in advance)

10. 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

11. Rinse in tap water 3 minutes.

12. Counterstain with Modified Harris Hematoxylin for 30 seconds.

13. Rinse in tap water until water is clear.

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

15. Dehydrate through the following solutions.

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

16. Coverslip
updated 09/16/04