Identification of IGF-1 receptor-beta in Formalin-Fixed, Paraffin Embedded Mouse Tissue

Reagents:

1X Automation Buffer

3% Hydrogen Peroxide

Antibody Diluent

Citrate Buffer

DAB Chromagen

Hematoxylin

Endogenous Blocking Solution: ImmunoPure Peroxidase Suppressor
Pierce
Rockford, IL 61105
www.piercenet.com
1-800-874-3723
Catalog #35000

Antibody Information:

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-001

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

Primary antibody : Rabbit anti-IGF-1R-Beta (H-60)
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog #sc-9038

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

Secondary antibody: Biotinylated Goat anti-rabbit IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #BA-1000

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: Mouse prostate

-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. 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

Buffer for 5 minutes

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

3. Quench endogenous peroxidase by placing slides in Immunopure Peroxidase Suppressor for 10 minutes.

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

5. Apply 10% Normal Goat Serum blocking serum and incubate 20 minutes.
Lot#_____ Reconstituted Date_____

DO NOT RINSE SLIDES, APPLY AVIDIN BIOTIN BLOCK

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 (Rabbit anti-IGF-1R-Beta) at a 1:200 dilution and incubate for one hour.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody anti-IGF-1R-Beta and use this to make the 1:200 dilution and incubate for one hour.
Lot#_____ Exp Date_____

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

8. Apply secondary antibody (Goat anti-rabbit) at a 1:600 dilution and incubate for 1 hour.
Lot#_____ Reconstitued Date_____

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

10. Apply pre-dilute Biotin-Streptavidin (Biogenex) and incubate for 15 minutes.
Lot#_____ Exp. Date_____

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_____

13. Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

16. 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% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip.

updated 02/25/04