Identification of IGF-1 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:

1X Automation Buffer

3% Hydrogen Peroxide

Antibody Diluent

Citrate Buffer

DAB Chromagen

Hematoxylin

Antibody Information

Santa Cruz Goat ABC Staining kit
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog #sc-2023
*This kit contains all reagents necessary to make blocking solution, secondary and label antibodies.

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

Primary antibody: Goat anti-IGF-1
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801

Negative Serum Control: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #005-000-121

Staining Procedure

-Positive Control Tissue: mouse skin (hair folicles)

-Stain localization: cytoplasmic

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

Buffer for 5 minutes

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

5. Block with Normal Donkey Serum for 1 hr at room temperature.
Made via 75 ul Block (blue cap) + 5 ml Diluent
Kit Lot#_____ Exp Date_____

DO NOT RINSE SLIDES.

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 (Goat anti-IGF-1) at 1:25 dilution and incubate for one hour.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal goat serum to the protein concentration of the primary antibody (IGF-1) and use this to make the 1:25 dilution. Apply normal goat serum to the slides and incubate for one hour.
Lot#_____ Reconstituted Date_____

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

9. Apply secondary antibody (Donkey anti-goat IgG) and incubate for 30 minutes.
Made via 75 ul NDS + 5 mls Diluent + 25 ul Donkey anti-Goat

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

11. Apply label complex and incubate for 30 minutes.
Made via 50 ul white cap + 50 ul purple cap + 2.5 ml diluent

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

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot#_____ exp_____ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. 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