Identification of Calgranulin 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-calgranulin
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog #sc-1496

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: tongue, macrophages

-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. Perform Heat Induced Epitope Retrieval using steamer.
Unmasking Techniques
Place slides in 1X Citrate Buffer and steam for 35 minutes.
Remove slides from steamer and cool for 20 minutes. Temp_____
Stop reaction by rinsing slides in D/W.
Place slides in 1X Automation buffer for 5 minutes.

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

5. Block with Normal Donkey Serum (1.5%) for 1 hr at room temperature.
Made via 75 ul Block (blue cap) + 5 ml Diluent
Kit Lot#_____ Exp Date_____

DO NOT RINSE SLIDES. CONTINUE TO 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.
No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Goat anti-calgranulin) at 1:1000 dilution and incubate for one hour at room temperature.

Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal goat serum to the protein concentration of the primary antibody and use this to make the 1:1000 dilution. Apply normal goat serum to the slides and incubate for one hour at room temperature.
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 Date_____ 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% ETOH	1 changes	3 minutes
100% ETOH	3 changes	3 minutes
xylene	2 changes	5 minutes

19. Coverslip

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