

Detection of Glucagon in Formalin-Fixed, Paraffin-Embedded, Rat Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information

Kit : Vector Rabbit Elite IgG ABC Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog: PK-6101

Note: The Vector Rabbit Elite Kit contains solutions needed to make the block, 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: Rabbit anti-glucagon (A565)

A565 recognizes alpha cells in the Islets of Langerhans in the pancreas. The antibody cross-reacts with glucagon in a large number of mammalian species.

Dako Corporation

Carpinteria, CA

www.dakousa.com

1-800-235-5763

Catalog #: A565

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

Staining Procedure

Positive Control Tissue: Pancreas -(Islets of Langerhans)

Stain Localization: Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	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 microwave oven.
Place a full rack of slides in tissue tek container containing 200ml of distilled water.
Microwave for 5 minutes at power level 5
Cool for 1 minute (Add more distilled water if necessary)
Microwave for 5 minutes at power level 5. Temp after Microwaving _____
Cool 20 minutes at room temperature.
Rinse in distilled water two times for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Apply block from Vector Rabbit Elite Kit and incubate for 20 minutes at room temperature.
Exp Date _____ New Kit: yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply the Avidin/Biotin Block
Lot# _____ Exp Date _____ New Kit: yes / no
Apply avidin block - 15 minutes at room temperature.
Quick rinse in 1X Automation Buffer. (Do not leave slides sitting in buffer)
Apply biotin block - 15 minutes at room temperature.
Wipe excess block.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE APPLYING PRIMARY ANTIBODY.

7. Apply primary antibody (Glucagon) at 1:3000 dilution and incubate for 30 minutes at room temperature.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of the normal rabbit serum to match the protein concentration of the primary antibody (Glucagon). Use this to make a 1:3000 dilution and incubate for 30 minutes at room temperature.

Lot# _____ Reconstituted Date _____

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

9. Apply secondary antibody from the Vector Rabbit Elite Kit and incubate for 30 minutes.

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

11. Apply Label antibody from Vector Rabbit Elite Kit and incubate for 30 minutes.
(Prepare at least 30 mins prior to use)

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 20 seconds.

16. Rinse in tap water until clear.

17. Gently agitate slides in 1X Automation buffer until they turn blue.

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 09/11/06