

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

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Staining Kit: Vectastain Elite ABC Kit (Rabbit IgG)

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-6101

Note: This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Polyclonal Rabbit Anti-Human Glucagon Antibody

Dakocytomation Corporation

Carpinteria, CA 93013

www.dako.com

1-800-235-5763

Code No. A0565

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 (alpha cells in the islets of Langerhans)
Stain Localization: Cytoplasmic

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.
3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Microwave

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer
(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Microwave for 5 minutes at power level 5.

Cool for 1 minute. (Add more citrate buffer, if necessary.)

Microwave again for 5 minutes at power level 5. *Temperature Before Cooling Slides* _____

Cool 20 minutes at room temperature.

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.

Vectastain Rabbit Elite Staining Kit

Exp. Date _____ New Kit: yes / no

6. Apply the block from the Rabbit Elite Kit. Incubate for 20 minutes at room temperature.

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

7. Avidin / Biotin Blocking Kit

Lot # _____ Exp. Date _____ New Kit: yes / no

Apply avidin block for 15 minutes at room temperature.

Quick rinse in 1X wash buffer.

Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:1000 dilution. Incubate for 30 minutes at room temperature.

Lot # _____ Exp. Date _____

For negative control slides, dilute normal rabbit serum so that its protein concentration matches that of

the primary antibody (if necessary). Then make a 1:1000 dilution. Apply the negative and incubate for 30 minutes at room temperature.

Lot # _____ Date Reconstituted _____

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the secondary antibody from Rabbit Elite Kit. Incubate for 30 minutes at room temperature.
11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. Apply the label complex from the Rabbit Elite Kit. Incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)
13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
14. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature. (Add 1 drop of DAB per ml of substrate)
Lot # _____ Exp. Date _____ New Kit: yes / no
15. Rinse the slides in tap water 3 minutes.
16. Counterstain with hematoxylin for 20 seconds.
17. Rinse the slides in tap water until water is clear.
18. Gently agitate slides in 1X wash buffer until the tissues turn blue.
19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

Updated 09/11/06