

Detection of Insulin in Formalin-Fixed, Paraffin-Embedded Rat 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 (Mouse IgG)

Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6102

Note: This kit contains all reagents necessary to make the blocking solution and secondary antibody.

Avidin / Biotin Blocking Kit

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

Primary Antibody: Mouse Monoclonal Anti-Insulin Antibody

Sigma-Aldrich
St. Louis, MO
www.sigmaaldrich.com
1-800-325-3010
Catalog # I2018

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum

BD Biosciences
San Jose, CA 95131
www.bdbiosciences.com
1-855-236-2772
Catalog # 557273

Staining Procedure

Positive Control Tissue: Pancreas – 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. Apply the block from the Mouse Elite Kit. Incubate for 20 minutes at room temperature.

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

5. 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.**

6. Apply primary antibody at a 1:8000 dilution. Incubate for 5 minutes at room temperature.
Lot # _____ Exp. Date _____

For negative control slides, dilute mouse IgG1 control serum so that its IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:8000 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 5 minutes at room temperature.

Lot # _____ Exp. Date _____

7. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
8. Apply the secondary antibody from Mouse Elite Kit. Incubate for 30 minutes at room temperature.
9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the label complex from the Mouse Elite Kit. Incubate for 30 minutes at room temperature.
(Prepare at least 30 minutes prior to use.)

11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. Apply the DAB chromogen and 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
13. Rinse the slides in tap water 3 minutes.
14. Counterstain with hematoxylin for 20 seconds.
15. Rinse the slides in tap water until water is clear.
16. Gently agitate slides in 1X wash buffer until the tissues turn blue.
17. 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

18. Coverslip

Updated 05/06/11