

# Detection of Somatostatin in Frozen Mouse Tissue

## **Reagent and Antibody Information**

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

[Rapid Fixx](#)

Staining Kit: Vectastain Elite ABC Kit (Rabbit IgG)

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://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](http://www.vectorlabs.com)

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Rabbit Anti-Human Somatostatin Antibody

Accurate Chemical and Scientific Corporation

Westbury, NY

[www.accuratechemical.com](http://www.accuratechemical.com)

1-800-645-6246

Catalog # MEDCLA439

Negative Control Serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 011-000-001

## Staining Procedure

Positive Control Tissue: Pancreas (Delta cells in the islets of Langerhans)  
Stain Localization: Cytoplasmic

1. Cut each frozen section at 6µm and mount on a positively-charged slide.  
Immediately fix the section in Rapid Fix Solution for 7 seconds.  
Rinse the slide thoroughly in tap water to remove excess fixative and then place in 1X Wash Buffer.  
Once all the slides have undergone this process, proceed to step 2.
2. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
3. Quench endogenous peroxidase by placing the slides in 0.3% hydrogen peroxide for 30 minutes.
4. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
5. Heat-Induced Epitope Retrieval Using The Decloaker  
Add 500 ml of distilled water to the pan inside the decloaker.  
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)  
Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* \_\_\_\_\_  
Depressurize for 10 minutes.  
Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* \_\_\_\_\_  
Rinse the slides in 2 changes of distilled water for 3 minutes each time.
6. 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

7. Apply the block from the Rabbit Elite Kit, and incubate for 20 minutes at room temperature.

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

8. 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 BUFFER.

9. Apply primary antibody at a 1:800 dilution, and incubate for 1 hour at room temperature.  
Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:800 dilution from this normalized serum, and apply to the slides.

Incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

10. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
11. Apply the secondary antibody from Rabbit Elite Kit, and incubate for 30 minutes at room temperature.
12. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
13. Apply the label complex from the Rabbit Elite Kit, and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)
14. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
15. Apply the DAB chromagen, 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
16. Rinse the slides in tap water 3 minutes.
17. Counterstain with Harris Hematoxylin for 20 seconds.
18. Rinse the slides in tap water until water is clear.
19. Gently agitate slides in 1X Wash Buffer until they turn blue.
20. Dehydrate through the following solutions:

| <b>Solutions</b> | <b>Repetitions</b> | <b>Time</b> |
|------------------|--------------------|-------------|
| 95% Ethanol      | 1 time             | 3 minutes   |
| 100% Ethanol     | 3 times            | 3 minutes   |
| Xylene           | 2 times            | 5 minutes   |

21. Coverslip

*Updated 08/23/07*