

# Detection of Synaptophysin in Formalin-Fixed, Paraffin-Embedded Mouse and Rat Tissue

## Reagents:

[1X Automation Buffer](#)  
[3% Hydrogen Peroxide](#)  
[Antibody Diluent](#)  
[Citrate Buffer](#)  
[DAB Chromagen](#)  
[Hematoxylin](#)

## Antibody Information

Blocking serum: Normal Goat Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #005-000-121

## 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-Synatophysin

Neomarkers/Labvision  
Fremont, CA  
[www.neomarkers.com](http://www.neomarkers.com)  
1-800-828-1628  
Catalog #RB-9044-P1

## 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

## Secondary antibody: Biotinylated Goat anti-rabbit IgG

Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666

Catalog# BA-1000

Label antibody: StriAviGen Super Sensitive Predilute Label Antibody

Biogenex Laboratories

San Ramon, CA 94583

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

1-800-421-4149

Catalog #HK330-5K

**Staining Procedure**

-Positive Control Tissue: pancreas: Islets of Langerhans

\*Note: Decrease stain intensity is observed in aged-slides (>2 months) and over-fixed tissues.

-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	3 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 decloaker.

Add 500 ml D/W to the pan of the decloaker.

Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.

Decloak for 5 minutes. Pressure\_\_\_\_\_

Depressurize for 10 minutes.

Remove pan top and cool for 10 min.Temp\_\_\_\_\_

Rinse in D/W, 2x for 3 min each

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

5. Block in 5% Normal Goat Serum for 20 minutes.

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

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.

Wipe excess block.  
DO NOT RINSE SECTIONS WITH BUFFER.

7. Apply primary antibody (Synaptophysin) at 1:500 dilution and incubate for 30mins.  
Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, normalize the protein concentration of normal rabbit serum to the protein concentration of the primary antibody and use this to make 1:500 dilution.  
Apply to slides and incubate for 30min.  
Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

9. Apply secondary antibody (biotinylated goat anti-rabbit) at 1:500 dilution and incubate for 30 minutes.  
Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

11. Apply Label (Biogenex) antibody and incubate for 30 minutes.  
Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_

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 water is 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