

# **Identification of Neuron-Specific Enolase in Formalin-Fixed, Paraffin-Embedded Mouse Tissue**

## **Reagents:**

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

## **Antibody Information**

Blocking Serum: Normal Horse Serum

Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390

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

1-800-367-5296

Catalog #008-000-001

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: Mouse anti-Neuron Specific Enolase

Lab Vision

Fremont, CA 94539

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

1-800-828-1628

Catalog#MS-1717-P1

Negative control serum: Normal Mouse Serum

Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390

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

1-800-367-5296

Catalog #015-000-001

Secondary antibody: Biotinylated Horse anti-Mouse IgG

Vector Laboratories, Inc.

Burlingame, CA 94010

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

1-800-227-6666

Catalog #BA-2001

Label antibody: Vector Standard Elite Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

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

1-800-227-6666

Catalog #PK-6100

**Staining Procedure**

-Positive Control Tissue: Pancreas: Islets of Langerhans

-Stain Localization: Cytoplasm

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	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. Perform Heat Induced Epitope Retrieval using Microwave Oven.  
Place a full rack of slides in container containing 200ml 1X citrate buffer.  
MWO for 5 minutes at power level 5  
Cool for 1 minute (Add 50ml citrate buffer to container)  
MWO for 5 minutes at power level 5. Temp after microwaving \_\_\_\_\_  
Cool 20 minutes at room temperature  
Rinse in distilled water 2 times for 3 minutes each
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Apply 10% Normal Horse Serum for 20 minutes at room temperature.

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 (NSE) at a 1:1500 dilution and incubate for one hour at room temperature.

Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (NSE) and use this to make a 1:1500 dilution. Apply and incubate for one hour at room temperature.

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

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

9. Apply Horse anti-mouse at 1:500 and incubate for 30 minutes at room temperature.

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

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

11. Apply Vector Elite label and incubate for 30 minutes at room temperature.

(Prepare at least 30 minutes prior to use)

Exp. Date \_\_\_\_\_ New Kit: yes / no

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 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

Updated 11/02/05