

# Detection of NeuN 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](#)

Blocking Serum: Normal Horse Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
www.jacksonimmuno.com  
1-800-367-5296  
Catalog # 008-000-001

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-NeuN Antibody  
Millipore  
Billerica, Massachusetts 01821  
www.millipore.com  
1-800-645-5476  
Catalog # AB1252  
**Lot # 2140038**

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum  
BD Biosciences  
San Jose, CA 95131  
www.bdbiosciences.com  
1-855-236-2772  
Catalog # 557273

Secondary Antibody: Biotinylated Horse Anti-Mouse IgG (H+L)  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # BA-2001

Label Complex: R.T.U. Vectastain Elite ABC Reagent  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
www.vectorlabs.com  
1-800-227-6666  
Catalog # PK-7100

### Staining Procedure

Positive Control Tissue: Uterus or mammary gland  
Stain Localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

<b>Solution</b>	<b>Repetitions</b>	<b>Time</b>
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 NxGen Decloaking Chamber™

Add 500 ml of distilled water to the pan inside the decloaker. All three of the decloaker's containers must be filled. Any containers without samples should have 250 ml of distilled water. The samples need to be in a container with a full rack of slides and about 200 ml of **1X citrate buffer**. (Insert blank slides into any empty slots in the rack to ensure even heating of slides.)

Decloak the slides for 15 minutes at 110°C. *Maximum Pressure* \_\_\_\_\_

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.

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

6. Block with 10% normal horse serum for 20 minutes at room temperature.

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

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

8. Apply primary antibody at a 1:2500 dilution. Incubate for 1 hour at room temperature.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, dilute mouse IgG1 control serum so that it's IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:2500 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 1 hour at room temperature.

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

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

10. Apply the horse anti-mouse secondary antibody at a 1:1000 dilution. Incubate for 30 minutes at room temperature.

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

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

12. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.

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

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

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 05/03/13*