

# Anti-NeuN, clone A60

Monoclonal Antibody

Cat. # MAB377

Lot # LV1519148

pack size: 500 µg

Store at 2-8°C

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS



## Certificate of Analysis

page 1 of 3

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IC, IH(P)	Av, Ch, Ft, H, M, Po, R, Pm, Sal	IgG <sub>1</sub>	N/A	M	46/48 kDa	N/A

### Background

NeuN antibody (NEUronal Nuclei; clone A60) specifically recognizes the DNA-binding, neuron-specific protein NeuN, which is present in most CNS and PNS neuronal cell types of all vertebrates tested. NeuN protein distributions are apparently restricted to neuronal nuclei, perikarya and some proximal neuronal processes in both fetal and adult brain although, some neurons fail to be recognized by NeuN at all ages: INL retinal cells, Cajal-Retzius cells, Purkinje cells, inferior olivary and dentate nucleus neurons, and sympathetic ganglion cells are examples (Mullen et al., 1992; Wolf et al., 1996). Immunohistochemically detectable NeuN protein first appears at developmental timepoints that correspond with the withdrawal of the neuron from the cell cycle and/or with the initiation of terminal differentiation of the neuron (Mullen et al., 1992). Immunoreactivity appears around E9.5 in the mouse neural tube and is extensive throughout the developing nervous system by E12.5. Strong nuclear staining suggests a nuclear regulatory protein function; however, no evidence currently exists as to whether the NeuN protein antigen has a function in the distal cytoplasm or whether it is merely synthesized there before being transported back into the nucleus. No difference between protein isolated from purified nuclei and whole brain extract on immunoblots has been found (Mullen et al., 1992).

### Presentation

Purified mouse immunoglobulin IgG<sub>1</sub> liquid in buffer containing 0.02 M phosphate buffer, 0.25 M NaCl, pH 7.6 with 0.1% sodium azide.

### Concentration

1 mg/mL

### Specificity

Vertebrate neuron-specific nuclear protein called NeuN (Neuronal Nuclei). Only one NeuN clone exists (A60) and reacts with an uncharacterized nuclear protein. MAB377 reacts with most neuronal cell types throughout the nervous system of mice including cerebellum, cerebral cortex, hippocampus, thalamus, spinal cord and neurons in the peripheral nervous system including dorsal root ganglia, sympathetic chain ganglia, and enteric ganglia. The immunohistochemical staining is primarily in the nucleus of the neurons with lighter staining in the cytoplasm. The few cell types not reactive with MAB377 include Purkinje, mitral and photoreceptor cells. Developmentally, immunoreactivity is first observed shortly after neurons have become postmitotic, no staining has been observed in proliferative zones. The antibody is an excellent marker for neurons in primary cultures and in retinoic acid-stimulated P19 cells. It is also useful for identifying neurons in transplants.

### Immunogen

Purified cell nuclei from mouse brain

### Molecular Weight

46/48 kDa

### Method of Purification

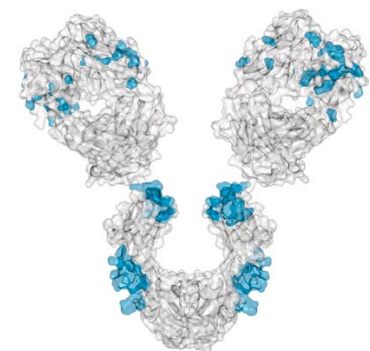
Protein A Purified

### Storage and Handling

Stable for 6 months at 2-8°C from date of receipt.

### Control

Positive control -Brain Tissue. Negative control - Any non neuronal tissue eg Fibroblasts



### References

1. Canola, Kriss, et al. (2007). *Invest Ophthalmol Vis Sci*. 48: 446-54.
2. Tippet, Lynette J., et al. (2007). *Brain*. 130: 206-221.
3. Rizzi, Simona, et al. (2007). *Hippocampus*. 17: 78-91.
4. Ladewig, Julia, et al. (2008). *Stem Cells*.
5. Mudo, G., et al. (2007). *Neuroscience*. 145: 470-83.

### Quality Control Testing

Routinely evaluated by immunohistochemistry on brain tissue.

### Immunohistochemistry(paraffin) Analysis:

NeuN (cat. # MAB377) staining pattern/morphology in rat cerebellum. Tissue pretreated with Citrate, pH 6.0. This lot of antibody was diluted to 1:100, using IHC-Select® Detection with HRP-DAB. Immunoreactivity is seen as nuclear staining in the neurons in the granular layer. Note that there is no signal detected in the nucleus of Purkinje cells.

**Optimal Staining With Citrate Buffer, pH 6.0, Epitope Retrieval: Rat Cerebellum**

**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence  
IH Immunohistochemistry (Tissue) IH(P) Immunohistochemistry (Paraffin)

**SPECIES LEGEND:** Av Avian Ch Chicken Ft Ferret H Human M Mouse Po Porcine (Pig) Pm Primate R Rat Rb Rabbit  
Sal Salamander WR Most Common Vertebrates

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## Additional Research Applications

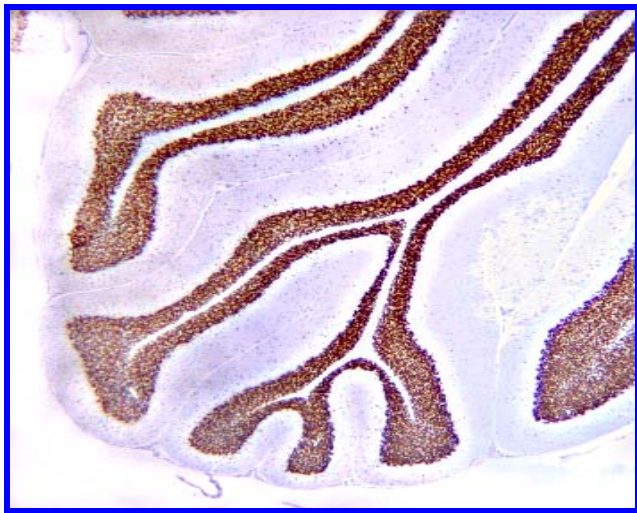
**Western Blot Analysis:** A previous lot of this antibody recognized 2-3 bands in the 46-48 kDa range and possibly another band at approximately 66 kDa.

**Immunocytochemistry:** 1:10-1:100 dilution from a previous lot was used. Neurons in culture should be permeabilized with 0.1% triton X-100. All primary antibody dilutions should be performed with simple solutions containing only buffer and primary antibody without excess protein blocks or detergents.

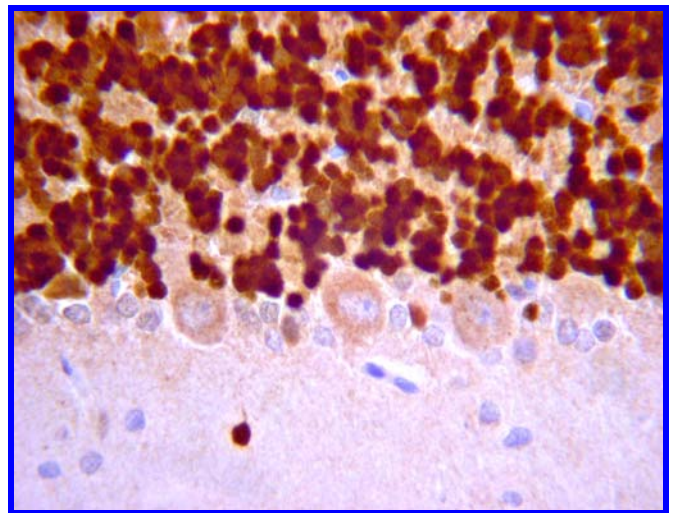
Optimal working dilutions must be determined by end user.

**Immunohistochemistry(paraffin) Analysis:**  
Representative images from a previous lot.

**Optimal Staining With Citrate Buffer, pH 6.0, Epitope Retrieval: Rat Cerebellum**



NeuN (cat. # MAB377) staining pattern/morphology in rat cerebellum. Tissue pretreated with Citrate, pH 6.0. A previous lot of antibody was diluted to 1:100, using IHC-Select<sup>®</sup> Detection with HRP-DAB. Immunoreactivity is seen as nuclear staining in the neurons in the granular layer. Low Magnification.



Mouse anti-NeuN (Catalog Number MAB377). Immunolocalization of NeuN (red) and BrdU (green) in the neurogenic regions of the mouse brain (dentate gyrus and subventricular zone). Photo courtesy of J.G. Emsley and T Hagg.

**PROTOCOL****Immunohistochemistry**

1. Use standard deparaffinization techniques on tissue specimens.
  2. Pretreat tissues using a citrate buffer, pH 6.0 and high heat epitope retrieval techniques.  
*Note: Do not allow tissues to dry out during the staining procedure.*
- The following steps are taken from the product manual for IHC Select<sup>®</sup> HRP/DAB Detection Kit (Cat. No. DAB050)*
3. Apply the blocking reagent to the tissue specimen and incubate in an enclosed chamber for 5 minutes.
  4. While holding the slide at a 45° angle, gently rinse the specimen with 1X Rinse Buffer for a minimum of 15 seconds. Tap the end of the slide onto a paper towel to remove excess Rinse Buffer.
  5. Apply a 1:100 dilution of primary antibody over the entire tissue specimen and incubate in an enclosed chamber at room temperature for 60 minutes.
  6. Rinse specimen as performed in Step 3.
  7. Apply the biotinylated secondary antibody to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
  8. Rinse specimen as performed in Step 3.
  9. Apply the Streptavidin-HRP solution to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
  10. Rinse specimen as performed in Step 3.
  11. Apply the DAB (chromogen reagent) to the tissue specimen and incubate in an enclosed chamber for 10 minutes.
  12. Rinse specimen as performed in Step 3.
  13. Apply the Hematoxylin counterstain solution to the tissue specimen and incubate in an enclosed chamber for 1 minute.
  14. Rinse specimen as performed in Step 3.
  15. Place the tissue slides directly into a container filled with deionized water until mounting.
  16. Mount a coverslip using an aqueous-based mounting media or for permanent mounting, dehydrate tissue through a graded series of alcohols, immerse in xylene, then apply a xylene-based mounting media (e.g. Permount) and coverslip.

**RELATED PRODUCTS (specific)**

cat #	description
MAB377B	■ Anti-NeuN, Clone A60, Biotin Conjugated
MAB377X	■ Anti-NeuN, clone A60, AlexaFluor <sup>®</sup> 488 conjugated
12-349	■ Goat Anti-Mouse IgG
AP124P	■ Goat anti-Mouse IgG, Peroxidase Conjugated, H+L

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 um
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0050	■ IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	■ Spray & Glow(TM) ECL Western Blotting 40 mL
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
2170	■ CHEMIBLOCKER-1LT
20-200	■ IMMUNOBLOT BLOCKING REAGENT 20G
17-500	■ CATCH AND RELEASE REVERSIBLE IMMUNOPRECIPITATION SYSTEM
16-266	■ PROTEIN G AGAROSE FAST FLOW 10 mL
16-125	■ PROTEIN A-AGAROSE 10 mL

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