

Anti-Nestin, clone rat-401

2018.1.8



Monoclonal Antibody

Cat. # MAB353

Lot # 2932512

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

pack size: 100 µg

Store at 2-8°C

Certificate of Analysis

page 1 of 2

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, IC, IH, IH(P)	M, R, not H	IgG1	N/A	<u>M</u>	200-220 kDa	P21263

Background

Nestin, a large intermediate filament protein (class Type VI) expressed during development and in myotendinous and neuromuscular junctions. Nestin expression is restricted, typically disappearing by E18. Nestin is thought to be a reasonable neuronal marker; however, recent studies have found nestin expression in other cell types such as endothelial cells (Mokry and Nemecek, 1998). Nestin identifies the most primitive neuroepithelium but also identifies many other embryonic tissues, so it is not specific for CNS. Nestin expression is seen in almost all GBMs (Glioblastoma multiformes) and many melanomas (both primary and metastatic) but not in any metastatic carcinomas.

Presentation

Purified immunoglobulin. Liquid in buffer containing 0.1M Tris-Glycine, 0.15M NaCl, 0.05% sodium azide, pH 7.4.

Concentration

1.0 mg/mL

Specificity

Rodent Nestin protein confirmed with rat and mouse. Rat-401 is not reactive with human nestin protein.

Immunogen

Nestin purified from embryonic rat spinal cord.

Molecular Weight

200-220 kDa

Storage and Handling

Maintain at 2-8°C undiluted aliquots for up to six months after date of receipt.

Control

Mouse embryonic brain tissue

Quality Control Testing

Immunohistochemistry(paraffin): Nestin (cat. # MAB353, Clone 401) staining of Mouse Pancreas, tissue was pretreated with citrate buffer, pH 6.0. A previous lot of this antibody was diluted to 1.25 µg/mL, using IHC-Select® detection with HRP-DAB. Immunoreactivity is detected in the islets of Langerhans.

Optimal Staining of Nestin Monoclonal Antibody: Mouse Pancreas

(See page 2)

Additional Research Applications

Western Blot: 200-220 kDa in reducing gels of newborn rat or mouse cell extracts. Note that nestin was originally reported (erroneously) to migrate at approximately 440 kDa by SDS-PAGE (Kachinsky, 1995).

For Westerns, prepare samples by boiling in 4 volumes of 125 mM Tris, pH 6.8, 10% 2-mercaptoethanol, 10% glycerol and 4.6% SDS. 5% PAGE gels are suggested. Block membranes with milk or BSA. Do not dilute primary antibody in buffer containing milk. Freezing and thawing of samples or samples not stored at -85°C will show breakdown of nestin sometimes into multiple fragments.

Immunohistochemistry: 1:20-1:200 dilution of a previous lot was used. Perfuse tissue with 4% paraformaldehyde at pH 7.4 for light microscopy or with either 4% paraformaldehyde at pH 10.0 or 4% paraformaldehyde with 0.1% glutaraldehyde at pH 7.4 for EM. Suitable for frozen or paraffin-



References

1. Mudo, G., et al. (2007). *Neuroscience*. 145: 470-83.
2. Okada, Seiji, et al. (2006). *Nat. Med.* 12: 829-34.
3. Muraguchi, Teruyuki, et al. (2007). *Nat. Neurosci.* 145: 470-83.
4. Aguado, Tania, et al. (2006). *J. Neurosci.* 26: 1551-61.
5. Palazuelos, Javier, et al. (2006). *FASEB J.* 20: 2405-7.

embedded sections.

Immunocytochemistry: 1:20-1:200 dilution of a previous lot was used. Fix cells in 4% paraformaldehyde buffered with 50 mM sodium borate at pH 9.5.

Optimal working dilutions and protocols must be determined by end user.

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

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

Please visit www.millipore.com for additional product information, test data and references.

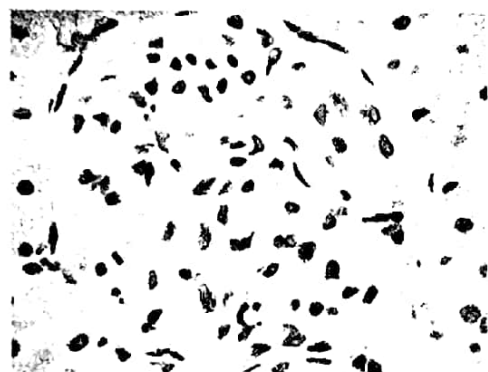
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Additional Research Applications

Immunohistochemistry(paraffin): Representative images from a previous lot.
Optimal Staining of Nestin Monoclonal Antibody: Mouse Pancreas



Nestin (cat. # MAB353, Clone 401) staining of Mouse Pancreas, tissue was pretreated with citrate buffer, pH 6.0. A previous lot of this antibody was diluted to 1.25 µg/mL, using IHC-Select® detection with HRP-DAB. Immunoreactivity is detected in the islets of Langerhans.



Negative control staining of Mouse Pancreas, tissue pretreated with citrate buffer, pH 6.0. Negative Control Reagent, using IHC-Select® Detection with HRP-DAB. No signal detected.

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

RELATED PRODUCTS (specific)

cat #	description
AB5922	■ Anti-Nestin
MAB5326	■ Anti-Nestin, human, clone 10C2
IHCR1006-6	■ Anti-Nestin, prediluted, clone rat-401
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 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
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 µm
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
2080	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
2170	■ CHEMBLOCKER-1LT
20-200	■ WESTERN BLOT BLOCKING REAGENT 20G

■ antibodies ■ Multiplex products ■ blottools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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