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Datasheet for 600-401-417

Nestin Antibody

Overview

Description:	Anti-Nestin (RABBIT) Antibody - 600-401-417
Item No.:	600-401-417
Size:	100 µg
Applications:	ELISA, IHC, WB
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	Nestin, is a large intermediate filament protein (class Type VI) that was first identified with a monoclonal antibody by Hockfield and McKay (1985). Nestin is expressed predominantly in stem cells of the central nervous system in the neural tube. Nestin is 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. Nestin expression is seen in almost all GBMs (Glioblastoma multiformes) and many melanomas (both primary and metastatic) but not in any metastatic carcinomas. Upon terminal neural differentiation, nestin is downregulated and replaced by neurofilaments.
Synonyms:	rabbit anti-Nestin antibody, rabbit anti-NES antibody, Nbla00170
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	NES
Reactivity:	Human, Mouse
Immunogen Type:	Conjugated Peptide

Immunogen:	Anti-Nestin affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 1484-1500 of human Nestin protein.
Purity/Specificity:	This affinity purified antibody is directed against human Nestin protein. The product was affinity purified from monospecific antiserum by immunoaffinity purification. A BLAST analysis was used to suggest cross reactivity with Nestin protein from human and Mesocricetus auratus (100% homology). Based on protein sequence homology also expect partial reactivity against Nestin homologues from mouse (93%) and rat (88%). Reactivity against homologues from other sources is not known.
Relevant Links:	• UniProtKB - P48681 • NCBI - 38176300 • GeneID - 10763

Application Details

Tested Applications:	ELISA, IHC, WB
Application Note:	Affinity purified Anti-Nestin has been tested for use in ELISA, Immunohistochemistry (IHC), and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 200-220 kDa in size corresponding to Nestin protein by western blotting in the appropriate cell lysate or extract.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:80,000
IHC:	5ug/ml
WB:	1:500 - 1:3,000

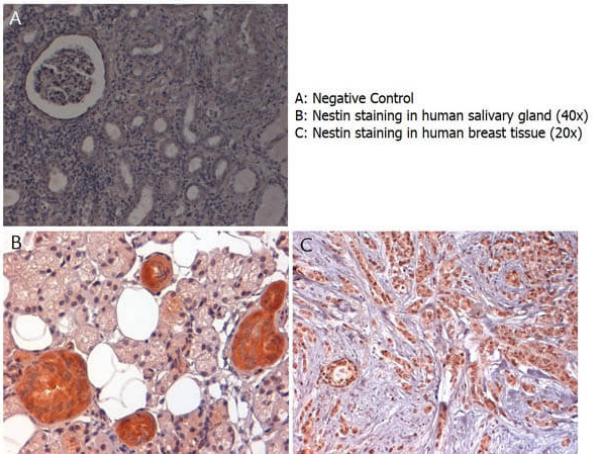
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.23 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

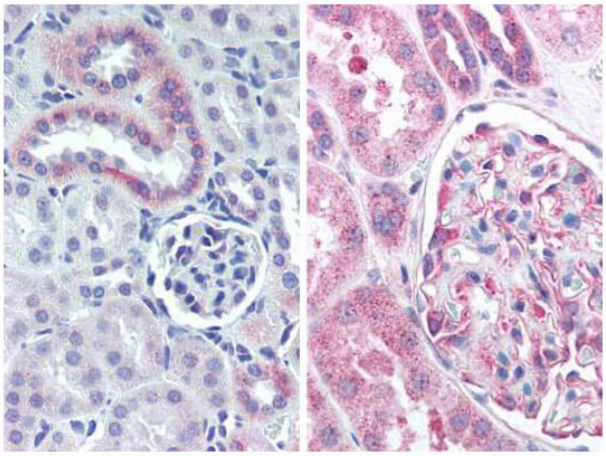
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store anti-Nestin at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

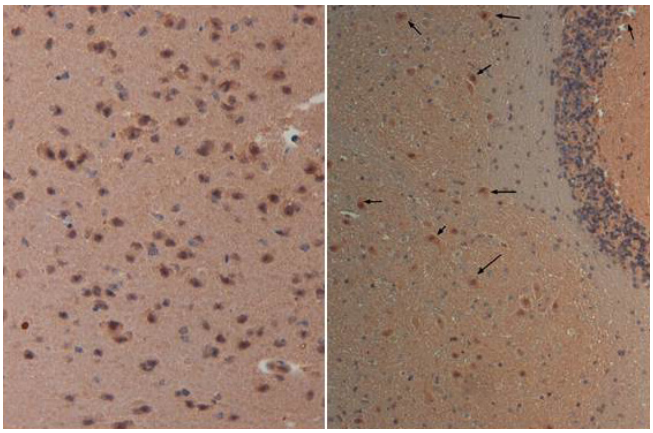
Images



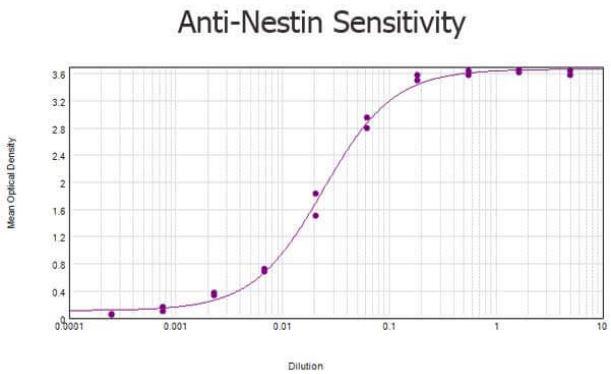
Immunohistochemistry
Immunohistochemistry with anti-nestin antibody showing nestin staining in cytoplasm of ductal epithelium of human salivary gland (B) and in nucleus and cytoplasm of human breast tissue (C). Formalin fixed/paraffin embedded sections were subjected to heat induced epitope retrieval (HIER) at pH 6.2 and then incubated with rabbit anti-nestin antibody at 4.0 µg/ml for 60 minutes. The reaction was developed using MACH 1 universal HRP polymer detection system and visualized with 3'3-diamino-benzidine substrate (DAB).



Immunohistochemistry
Immunohistochemistry with Anti-Nestin antibody. Tissue: mouse kidney (Left) and human kidney (Right). Fixation: formalin-fixed, paraffin-embedded tissue. Antigen retrieval: heat-induced. Primary antibody: 5 µg/ml. Staining: antibody as precipitated red signal with a hematoxylin purple nuclear counterstain.

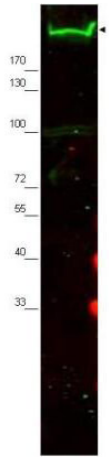


Immunohistochemistry
Immunohistochemistry with Anti-nestin antibody at 40X (left) and 20X (right) Tissue: Brain and Cerebellum (right) Fixation: FFPE buffered formalin 10% conc Antigen retrieval: Heat, Citrate pH 6.2. Pressure Cooker Primary antibody: 20ug/ml 1 hour @ room T Secondary antibody: Goat anti Rabbit Polymer HRP Prediluted by the manufacturer 30 min. @ room T Staining: antibody as precipitated red signal with a hematoxylin purple nuclear counterstain.



ELISA
ELISA results of purified Rabbit anti-Nestin Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1µg of conjugate. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% fish gel, Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) (p/n 611-103-122) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).

Western Blot
Western blot using Rockland's Affinity Purified anti-Nestin antibody shows detection of a band ~220 kDa corresponding to human Nestin (arrowhead). Undifferentiated HCN-1A human brain cortical neuron neuronal progenitor lysate (lane 1), or differentiated HCN-1A human brain cortical neuron neuronal progenitor lysate (lane 2) were separated by SDS-PAGE using 4-20% gradient gel. After transfer onto nitrocellulose, the membrane was blocked and then probed with the primary antibody diluted to 1:2,000 overnight at 4°C. The membrane was then washed and reacted with a 1:10,000 dilution of peroxidase conjugated affinity purified Gt-a-Rabbit IgG [H&L] MX (611-1302) for 45 min at room temperature. Image was captured using film. Other detection systems will yield similar results. Image courtesy of Prof. F.H. Gage of the Salk Institute, San Diego, CA



Western Blot

Western blot using Rockland's Affinity Purified anti-Nestin antibody shows detection of a band ~220 kDa corresponding to mouse Nestin (arrowhead). Approximately 30 g of MEF whole cell lysate was separated by SDS-PAGE using a 4-20% gradient gel. After transfer onto nitrocellulose, the membrane was blocked and then probed with the primary antibody diluted to 1:2,000 overnight at 4°C. The membrane was then washed and reacted with a 1:10,000 dilution of IRDye™800 conjugated Gt-a-Rabbit IgG [H&L] MX (611-132-122) for 45 min at room temperature. IRDye800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

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