Product Datasheet

Vimentin Antibody
NB300-223-0.5ml

Unit Size: 0.5 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Reviews: 6 Publications: 70

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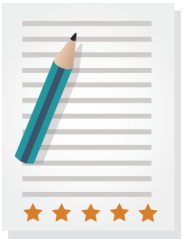
Updated 10/23/2024 v.20.1



NB300-223-0.5ml

Vimentin Antibody

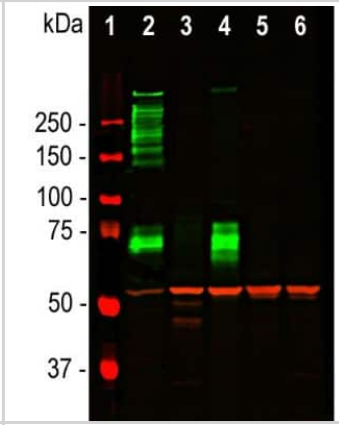
Product Information	
Unit Size	0.5 ml
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.035% Sodium Azide
Isotype	IgY
Purity	IgY purified
Buffer	Supplied as concentrated total IgY preparation from egg yolk. Exact concentration of target specific IgY is not quantifiable as the preparation contains both immune IgY specific for the target and also irrelevant, non-immune IgY.
Target Molecular Weight	53.6 kDa

Product Description	
Description	For purification, first organic extraction is performed to remove the lipid and proteolipds. Then ammonium sulfate precipitation of the aqueous is done to precipitate and concentrate the IgY fraction. Finally, extensive dialysis is performed against PBS followed by addition of Sodium Azide.
Host	Chicken
Gene ID	7431
Gene Symbol	VIM
Species	Human, Mouse, Rat, Porcine, Bovine, Canine, Chicken, Equine
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:33675257).
Marker	Mesenchymal Cells Marker
Immunogen	Full length recombinant human Vimentin Antibody expressed in and purified from E. coli. [Swiss-Prot# P08670]
Notes	Chicken products cannot be exported to Canada.

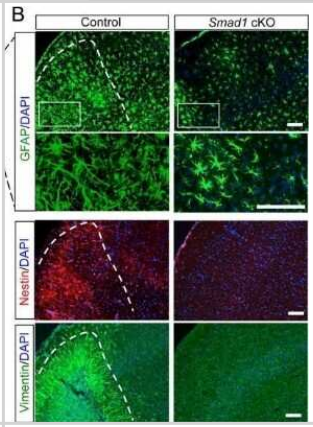
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:10000-1:20000, Immunohistochemistry 1:150, Immunocytochemistry/ Immunofluorescence 1:5000, Immunohistochemistry-Paraffin 1:150, Immunohistochemistry-Frozen
Application Notes	Use in IHC-P and IHC-Fr reported in scientific literature (PMID: 23752194 and 25626686).

Images

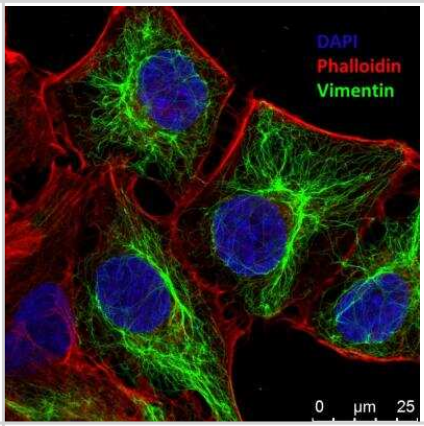
Western Blot: Vimentin Antibody [NB300-223] - Analysis of tissue and cell lysates. Antibody at 1:5000 in red. [1] protein standard (red), [2] rat whole brain lysate, [3] HeLa, [4] SH-SY5Y, [5] HEK293, and [6] NIH-3T3 cell lysates. NB300-223 binds to the vimentin protein showing a single band at ~50 kDa. The blot was simultaneously probed with mouse mAb to MAP2C/D, dilution 1:5000 in green, revealing multiple bands around 280 kDa that correspond to full length MAP2A/2B isotypes, and ~70 kDa bands which are MAP2C/D isotypes. MAP2 isotypes are seen only in extracts containing neuronal lineage cells.



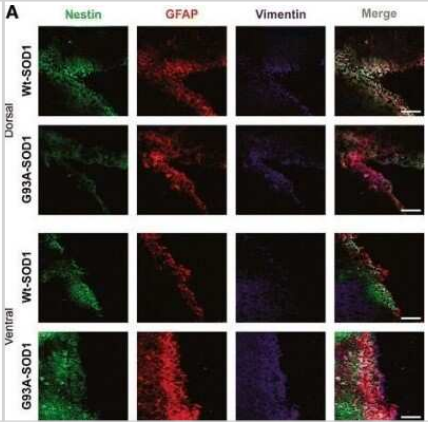
Immunohistochemistry: Vimentin Antibody [NB300-223] - Attenuated reactive astrocytosis after stroke in Smad1 cKO mice. Enlarged IHC images of boxed areas at the cortical peri-infarct area with the indicated reactive astrocyte markers GFAP, Nestin, and Vimentin. Enlarged images of GFAP IHC highlight the hypertrophic morphology of GFAP+ astrocytes in mutants. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0136967), licensed under a CC-BY license.



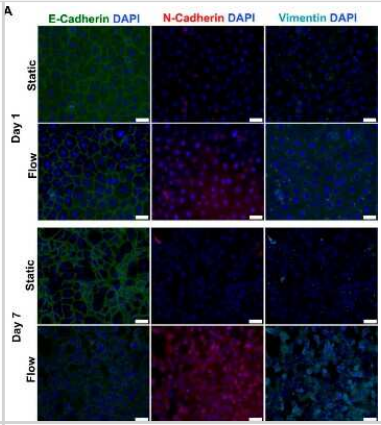
Immunocytochemistry/Immunofluorescence: Vimentin Antibody [NB300-223] - Human A7 cells. Cells were fixed with 10% formalin and permeabilized with 0.1% TritonX-100. After blocking, cells were incubated with primary antibody (1:600) at 4C O/N followed with Alexa Fluor 488 labeled secondary antibody and counterstained with DAPI and Phalloidin for nuclei and F-actin respectively. Imaged with Leica TCS SP5 confocal microscope. ICC/IF image submitted by a verified customer review.



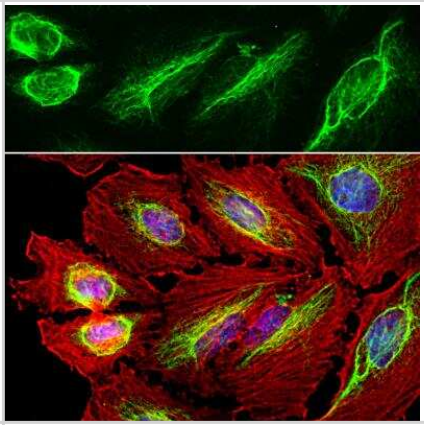
Immunohistochemistry: Vimentin Antibody [NB300-223] - Increased numbers of differentiating NSPCs in SVZ of G93A-SOD1 brain. Confocal microscopy images of dorsal and ventral regions of SVZ in G93A-SOD1 and Wt-SOD1 brain at postnatal week 18, stained for nestin (green), GFAP (red), and vimentin (blue). Scale bar = 50 um. Image collected and cropped by CiteAb from the following publication (https://www.molecularbrain.com/content/8/1/5), licensed under a CC-BY license.



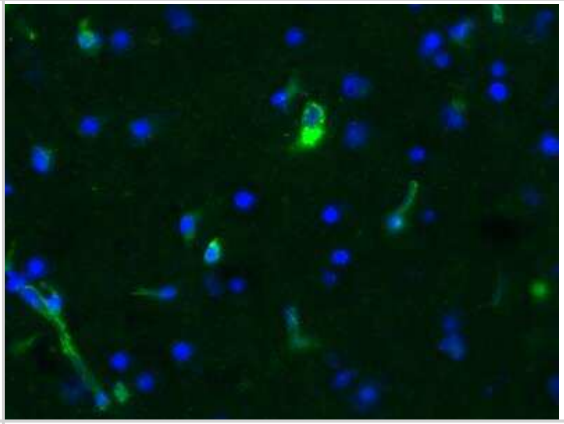
Immunocytochemistry/Immunofluorescence: Vimentin Antibody [NB300-223] - Comparative analysis of epithelial and mesenchymal markers expressions. Fluorescence immunocytochemistry staining for E-cadherin (green), N-cadherin (red) and vimentin (cyan) was performed on esophageal cancer cells under static and microfluidic flow conditions at different time points. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep38221), licensed under a CC-BY license.



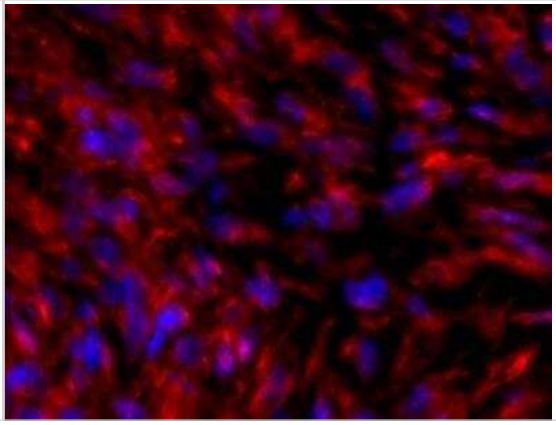
Immunocytochemistry/Immunofluorescence: Vimentin Antibody [NB300-223] - Analysis of HeLa cell culture. Antibody at 1:10,000 in green, and costained with mouse mAb to actin, dilution 1:500 in red. The blue is DAPI staining of nuclear DNA. The vimentin antibody stains the intermediate filament network while the actin antibody labels the submembranous cytoskeleton, stress fibers, and bundles of actin associated with cell adhesion sites.



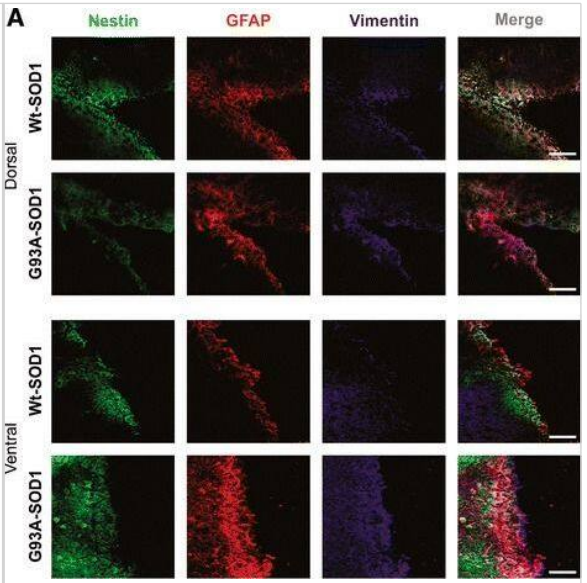
Immunohistochemistry-Frozen: Vimentin Antibody [NB300-223] - 10 um Cryosections from PFA fixed murine brain acute slices stained with Vimentin antibody (1:600) and Alexa Fluor 488 anti chicken IgG. Antibody showed specific staining, also of blood vessels. IHC-Fr image submitted by a verified customer review.



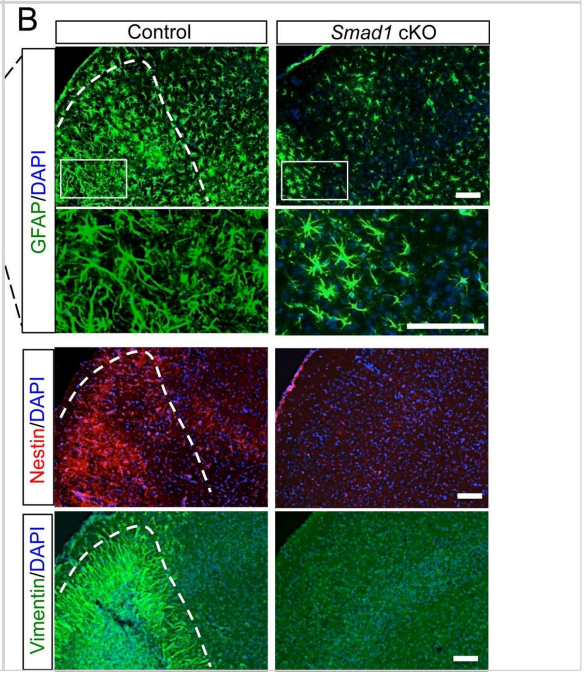
Immunohistochemistry-Frozen: Vimentin Antibody [NB300-223] - Infarcted mouse heart frozen sections stained with vimentin antibody at 1:200. Secondary antibody at 1:500. Fixation with 4% PFA. IHC-Fr image submitted by a verified customer review.



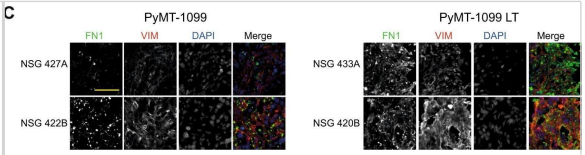
Immunocytochemistry/ Immunofluorescence: Vimentin Antibody [NB300-223] - Increased numbers of differentiating NSPCs in SVZ of G93A-SOD1 brain. (A) Confocal microscopy images of dorsal & ventral regions of SVZ in G93A-SOD1 & Wt-SOD1 brain at postnatal week 18, stained for nestin (green), GFAP (red), & vimentin (blue). Scale bar = 50 µm. (B) Quantification of nestin-, GFAP-, & vimentin-positive cells in SVZ of G93A-SOD1 & Wt-SOD1 mice. Data are means ± SD of 3 mice per group. **p < 0.001; limma moderated t-test. (C) Hematoxylin & eosin staining of SVZ sections adjacent to those analyzed by confocal microscopy. Scale bar = 50 µm. D: dorsal. V: ventral. (D) Confocal microscopy images showing Dlx2-stained cells in ventral SVZ in G93A-SOD1 & Wt-SOD1 brain. Scale bar = 50 µm. (E) Quantification of Dlx2-positive cells in G93A-SOD1 & Wt-SOD1 SVZ. Data are means ± SD of 3 mice per group. **p < 0.001; limma moderated t-test. Image collected & cropped by CiteAb from the following publication (https://molecularbrain.biomedcentral.com/articles/10.1186/s13041-015-0095-0), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Vimentin Antibody [NB300-223] - Attenuated reactive astrocytosis after stroke in Smad1 cKO mice. (A) Images of IHC for reactive astrocyte marker GFAP on ipsilateral hemisphere at 7 days (top two panels) or 3 months (bottom panels) post-stroke. The border between the stroke core & peri-infarct area is outlined by dotted lines. Arrows point to striatal infarct core. (B) Enlarged IHC images of boxed areas in (A) at the cortical peri-infarct area (blue box in D) with the indicated reactive astrocyte markers GFAP, Nestin, & Vimentin. Enlarged images of GFAP IHC highlight the hypertrophic morphology of GFAP+ astrocytes in mutants. (C) Quantification of the number of GFAP+ astrocytes & the intensity of GFAP immunoreactivity (IR) at the peri-infarct area shown in (B). n = 4, one-way ANOVA for the number of astrocytes, unpaired Student's t-test for GFAP intensity, ***p<0.001, Ipsi, ipsilateral; Ctra, contralateral cortex. (D) Diagram of infarct territory affected by tMCAO (blue) & peri-infarct cortical area (blue box). (E) Reactive astrocytosis was similarly attenuated in the ipsilateral hippocampus of Smad1 cKO mice. Scale, 500 µm (A), 100 µm (B), & 200 µm (E). Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0136967), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Vimentin Antibody [NB300-223] - Tumor formation & metastasis by PyMT-1099 cells. (A) PyMT-1099 cells untreated or treated with TGFβ for >20 days (PyMT-1099 LT) were injected orthotopically into mammary fat pads of NSG mice. The graph represents tumor growth in PyMT-1099 & PyMT-1099 LT group of mice. (B) Histological tumor sections from tumors of PyMT-1099 or PyMT-1099 LT cells described in (A) were stained with H&E to assess the morphology of primary tumors. Representative microphotographs are shown from tumors of 2 out of the 6 mice used in the experiment. (C) Immunofluorescence analysis was performed to assess the expression of the EMT markers FN1, VIM, E-CAD & N-CAD in tumors formed by PyMT-1099 or PyMT-1099 LT cells in the experiment described in (A). DAPI was used as a nuclear counterstain. Representative pictures are shown from tumors of 2 out of the 6 mice used in the experiment. Scale bar, 100 µm. (D) The graph represents the number of lung metastases formed in NSG mice orthotopically transplanted with PyMT-1099 or PyMT-1099 LT cells; n = 6. (E) The graph represents the number of lung metastases formed in NSG mice injected with PyMT-1099 or PyMT-1099 LT cells through the tail vein; n = 6. The mice were sacrificed 8 weeks post-injection, & lungs were resected for the analysis of cancer cell colonization/ metastases formation. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30108334), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Autumn B. Morgan, Yan Fan, Denise M. Inman The ketogenic diet and hypoxia promote mitophagy in the context of glaucoma Frontiers in Cellular Neuroscience 2024-05-22 [PMID: 38841201]

Zrelski MM, Hösele S, Kustermann M et al. Plectin deficiency in fibroblasts deranges intermediate filament and organelle morphology, migration, and adhesion The Journal of investigative dermatology 2023-09-14 [PMID: 37716646]

Gupta K, Chen D, Wells RG Microcystin-RR is a biliary toxin selective for neonatal cholangiocytes bioRxiv : the preprint server for biology 2023-08-13 [PMID: 37609158] (ICC/IF)

Suprewicz ?, Swoger M, Gupta S et al. Extracellular Vimentin as a Target Against SARS-CoV-2 Host Cell Invasion Small 2022-02-01 [PMID: 34866333]

Dai DL, Li M, Lee EB. Human Alzheimer's disease reactive astrocytes exhibit a loss of homeostastic gene expression Acta Neuropathologica Communications 2023-08-02 [PMID: 37533101] (Immunohistochemistry-Paraffin)

Orzechowska-Licari EJ, Bialkowska AB, Yang VW Sonic Hedgehog and WNT signaling regulate a positive feedback loop between intestinal epithelial and stromal cells to promote epithelial regeneration Cellular and molecular gastroenterology and hepatology 2023-07-20 [PMID: 37481204]

Details:
1:400 WB dilution

Jassim AH, Nsiah NY, Inman DM. Ocular Hypertension Results in Hypoxia within Glia and Neurons throughout the Visual Projection Antioxidants (Basel) 2022-04-29 [PMID: 35624752]

Uppal S, Postnikova O, Villasmil R et al. Low-Density Lipoprotein Receptor (LDLR) Is Involved in Internalization of Lentiviral Particles Pseudotyped with SARS-CoV-2 Spike Protein in Ocular Cells International journal of molecular sciences 2023-07-24 [PMID: 37511618] (B/N)

Ostrowska-Podhorodecka Z, Ali A, Norouzi M et al. Vimentin-mediated myosin 10 aggregation at tips of cell extensions drives MT1-MMP-dependent collagen degradation in colorectal cancer FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2023-08-01 [PMID: 37440280]

Vos S, Aaron R, Weng M et al. CD40 Upregulation in the Retina of Patients With Diabetic Retinopathy: Association With TRAF2/TRAF6 Upregulation and Inflammatory Molecule Expression Investigative ophthalmology & visual science 2023-06-01 [PMID: 37294707] (Immunohistochemistry, Human)

Kálmán M, Oszwald E, Pócsai K Three-plane description of astroglial architecture and gliovascular connections of area postrema in rat: Long tanycyte connections to other parts of brainstem The Journal of comparative neurology 2023-06-01 [PMID: 36994627] (ICC/IF, Rat)

Feng W, Bais A, He H et al. Single-cell transcriptomic analysis identifies murine heart molecular features at embryonic and neonatal stages Nature communications 2022-12-27 [PMID: 36575170] (IHC, Mouse)

Details:
Dilution used in IHC 1:500

More publications at http://www.novusbio.com/NB300-223

Procedures

Immunostaining of cells in tissue culture protocol specific for Vimentin Antibody (NB300-223)

Immunostaining of cells in tissue culture

The purpose of fixation is to denature the components of cells enough so that they stay on the dish and can be bound by antibodies, hopefully without destroying cellular morphology. Fixatives such as formalin, paraformaldehyde and glutaraldehyde chemically cross-link proteins, by binding to amino acid side chains. This chemical modification can also have the consequence of blocking antibody binding sites. Substances such as acetone and methonal are not true fixatives but are denaturants, which precipitate proteins without covalently modifying them. We routinely use a combination of mild formalin fixation followed by cold methanol for neurons, mixed neuron/glial cultures and most used cell lines. The formalin preserves the cellular morphology quite well, while the methanol further denatures the proteins of the cells and helps keep what is left of the cell adherent to the dish. For soluble proteins it may be necessary to skip the methanol step, but in this case you have to be very careful with the washing steps, as the cells tend to wash off the dish. Certain antibodies may be very sensitive to formalin fixation, so you may have to experiment a little, perhaps skipping that step. The following procedure work for antibodies to most cytoskeletal and signaling molecules. This procedure is good for cells in 6 well dishes or in 35mm tissue culture plates.

1. Draw the culture medium with aspirator and add 1 ml of
3.7 % formalin in PBS solution to the dish (from 10mls Fisher 37% formalin plus 90mls PBS, the Fisher formalin contains 37% formaldehyde plus about 1% methanol which may be relevant sometimes). Let sit at room temp for 1 minute (can add 0.1% Tween 20 to PBS used here and all subsequent steps to reduce background; probably best not to do this first time around, though, as it may extract your antigen or help wash your cells off the dish).
2. Take off the formalin/PBS and add 1ml of cold methanol (-20C, kept in well sealed bottle in fridge). Let sit for no more than 1 minute.
3. Take off methanol and add 1ml of PBS, not letting the specimen dry out. To block nonspecific antibody binding can add ~10ml (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Then add antibody reagents. Typically 100ml of hybridoma tissue culture supernatent or 1ml of mouse ascites fluid or crude serum. Incubate for 1 hour at room temp. (or 37C for 30 minutes to 1 hour, or 4C overnight, exact time not too critical). Shake gently for well adherent cell lines (3T3, HEK293, etc.).
4. Remove primary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.
5. Add 0.5 mls of secondary antibody. These are fluorescently labeled goat anti-chicken antibodies and are conjugated to ALEXA dyes and are from Molecular probes (Eugene Oregon, the ALEXA dyes are sulphonated rhodamine compounds and are much more stable to UV than FITC, TRITC, Texas red etc.). Typically make 1:2,000 dilutions of these secondaries in PBS plus 1% goat serum, BSA or non fat milk carrier. Incubate for 1 hour at room temp. (or 37C for 30 minutes to 1 hour, or 4C overnight). Shake gently for well adherent cell lines (3T3, HEK293, etc.).
6. Remove secondary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.
7. Drop one drop of Fisher mounting medium onto dish and apply 22mm square coverslip. View in the microscope.

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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