Product Datasheet

Vimentin Antibody
NB300-223SS

Unit Size: 0.025 ml

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

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Publications: 11

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NB300-223SS

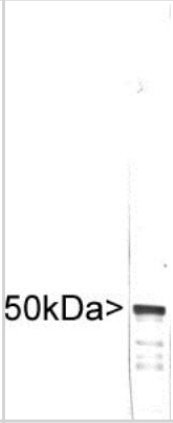
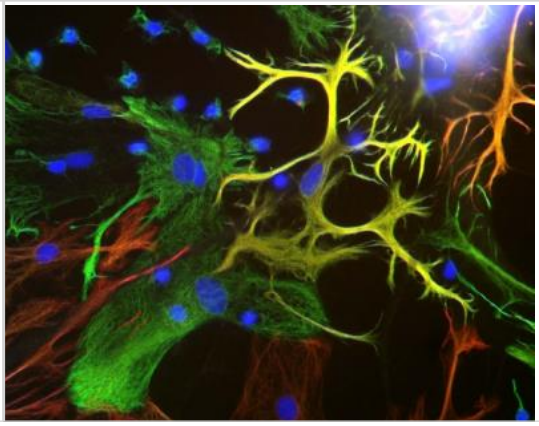
Vimentin Antibody

Product Information	
Unit Size	0.025 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	5mM Sodium Azide
Isotype	IgY
Purity	Ammonium sulfate precipitation
Buffer	PBS
Target Molecular Weight	50 kDa

Product Description	
Host	Chicken
Gene ID	7431
Gene Symbol	VIM
Species	Human, Mouse, Rat
Species Reactivity	Human, mouse, and rat.
Marker	Mesenchymal Cells Marker
Immunogen	Recombinant human Vimentin purified from E. coli. [Swiss-Prot# P08670]

Product Application Details	
Applications	Western Blot, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Immunohistochemistry-Paraffin 1:150, Western Blot 1:10000-1:20000, Immunocytochemistry/Immunofluorescence 1:5000, Immunohistochemistry 1:150
Application Notes	This Vimentin antibody is useful for Immunohistochemistry paraffin embedded sections, Western blot and Immunocytochemistry/Immunofluorescence.

Images

Western Blot: Vimentin Antibody [NB300-223] - Western blot of crude extract of human embryonic kidney Hek293 cells stained with NB300-223, showing a single strong clean band at approx. 50kDa.	
Immunocytochemistry/Immunofluorescence: Vimentin Antibody [NB300-223] - View of mixed neuron/glial cultures stained with NB300-223 (green) and rabbit antibody to GFAP antibody NB300-141(red). Vimentin is expressed alone in fibroblastic and endothelial cells, which are the flattened cells in the middle of the image which appear green. Astrocytes may express primarily GFAP, or GFAP and vimentin, and so appear red (GFAP only) or golden yellow (GFAP and Vimentin). In cells which express both GFAP and vimentin, the two protein assemble to produce heteropolymer filaments.	

Publications

Marcuzzo S, Bonanno S, Kapetis D et al. Up-regulation of neural and cell cycle-related microRNAs in brain of amyotrophic lateral sclerosis mice at late disease stage Mol Brain. 2015 Jan 28 [PMID: 25626686] (IHC-Fr, Mouse)

Phamduong E, Rathore MK, Crews NR et al. Acute and Chronic Mu Opioids Differentially Regulate Thrombospondins 1 and 2 Isoforms in Astrocytes. ACS Chem Neurosci 2013 Dec 5 [PMID: 24304333] (ICC/IF, Rat)

Ponzo M, Lesurf R, Petkiewicz S et al. Met induces mammary tumors with diverse histologies and is associated with poor outcome and human basal breast cancer. Proc Natl Acad Sci U S A 2009 [PMID: 19617568]

Murray ME, Mendez MG, Janmey PA et al. Substrate Stiffness Regulates Solubility of Cellular Vimentin. Mol Biol Cell. 2013 Oct 30 [PMID: 24173714] (ICC/IF, WB, Human)

Stadler SC, Vincent CT, Fedorov VD et al. Dysregulation of PAD4-mediated citrullination of nuclear GSK3beta activates TGF-beta signaling and induces epithelial-to-mesenchymal transition in breast cancer cells. Proc Natl Acad Sci U S A 2013 Jul 16 [PMID: 23818587] (WB, ICC/IF, Human)

Busch SE, Moser RD, Gurley KE et al. ARF inhibits the growth and malignant progression of non-small-cell lung carcinoma. Oncogene 2013 Jun 10 [PMID: 23752194] (IHC-P, Mouse)

Muir AB, Lim DM, Benitez AJ et al. Esophageal epithelial and mesenchymal cross-talk leads to features of epithelial to mesenchymal transition in vitro Exp Cell Res 2012 Dec 10 [PMID: 23237990] (IHC, ICC/IF, Human)

Rankin, E et al. Renal Cyst Development in Mice with Conditional Inactivation of the von Hippel-Lindau Tumor Suppressor. Cancer Res; 66: (5). 2006 [PMID: 16510575] (IHC, Mouse)

Johnstone, C N et al. Inhibits Breast Cancer Tumorigenicity Promotes CDK9-Mediated Peroxisome Proliferator-Activated Receptor Gamma 1 Phosphorylation. Mol Cell Biol; 28: 687 - 704. Jan-08. [PMID: 17998334] (IHC, Human)

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA. Genomic analysis of reactive astrogliosis. J Neurosci;32(18):6391-410. 2012 May 2. [PMID: 22553043] (IHC, ICC/IF, Mouse)

Okawa T, Michaylira CZ, Kalabis J et al. The functional interplay between EGFR overexpression, hTERT activation, p53 mutation in esophageal epithelial cells with activation of stromal fibroblasts induces tumor development, invasion, differentiation. Genes amp; Dev, 2007; 21(21):2788-803. 2007 November 1. [PMID: 17974918] (WB, Human)

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.

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