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Vimentin Polyclonal Antibody

Catalog Number **PA1-10003**

Product data sheet

Details	
Size	100 µL
Host/Isotope	Chicken / IgY
Class	Polyclonal
Type	Antibody
Immunogen	Full length recombinant human vimentin
Conjugate	Unconjugated
Form	Liquid
Concentration	Conc. Not Determined
Storage buffer	PBS
Contains	0.02% sodium azide
Storage Conditions	4° C

Species Reactivity	
Species reactivity	Bovine, Chicken, Horse, Human, Mouse, Pig, Rat
Published species	Rat, Human, Mouse, Not Applicable

Tested Applications	Dilution *
Western Blot (WB)	1:10,000
Immunocytochemistry (ICC/IF)	1:5,000

Published Applications	
Immunocytochemistry (ICC/IF)	See 1 publications below
Miscellaneous PubMed (Misc)	See 1 publications below
Immunohistochemistry (IHC)	See 3 publications below
Western Blot (WB)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

PA1-10003 can be used to study stem cells and generally to reveal the intermediate filament cytoskeleton. The immunogen used to generate this antibody was full length recombinant human vimentin. The antibody works well on all mammals tested to date, and it was generated in chicken by standard procedures and immunoglobulin was extracted from egg yolk.

Background/Target Information

Vimentin is a developmentally regulated intermediate filament protein (IFP) found in cells of mesenchymal origin. It is believed to be involved with the intracellular transport of proteins between the nucleus and plasma membrane. Unlike other IFP proteins, vimentin is expressed, along with desmin, during the early stages of cellular development. During the development process, vimentin is exchanged for new, tissue-specific IFPs. Vimentin has been implicated to be involved in the rate of steroid synthesis via its role as a storage network for steroidogenic cholesterol containing lipid droplets. Vimentin phosphorylation by a protein kinase causes the breakdown of intermediate filaments and activation of an ATP and myosin light chain dependent contractile event. This results in cytoskeletal changes that facilitate the interaction of the lipid droplets within mitochondria, and subsequent transport of cholesterol to the organelles leading to an increase in steroid synthesis.

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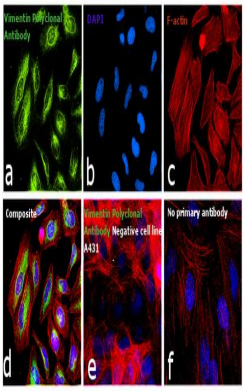
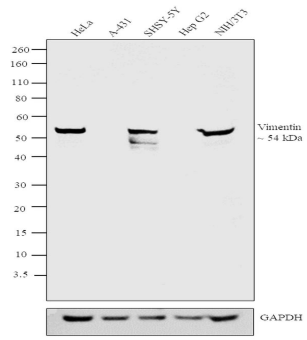
Product Images For Vimentin Polyclonal Antibody



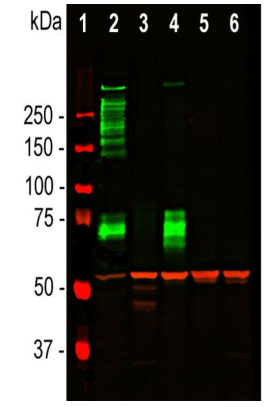
Vimentin Antibody (PA1-10003)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. Immunofluorescence analysis using Vimentin Polyclonal Antibody (Product # PA1-10003) showed expression of Vimentin in HeLa compared to A-431. {RE}



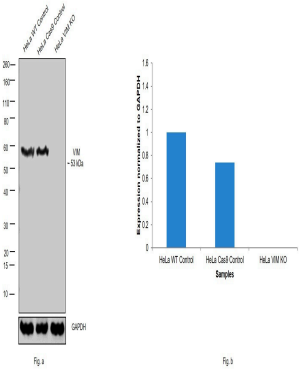
Vimentin Antibody (PA1-10003) in WB

Western blot analysis was performed on Whole cell extracts (30 µg lysate) of HeLa (Lane 1), A-431 (Lane 2), SHSY-5Y (Lane 3), Hep G2 (Lane 4) and NIH/3T3 (Lane 5). The blot was probed with Anti-Vimentin Polyclonal Antibody (Product # PA1-10003, 1:2000 dilution) and detected by chemiluminescence using Goat anti-Chicken IgY (H+L) Secondary Antibody, HRP (Product # A16054, 0.25 µg/ml, 1:4000 dilution). A 54 kDa band corresponding to Vimentin was observed across all the cell lines positive for Vimentin (Lanes 1, 3 and 5), while this band was absent in the cell lines which do not express Vimentin protein (Lanes 2 and 4).



Vimentin Antibody (PA1-10003) in WB

Western blot analysis of a Vimentin in tissue and cell lysates using a Vimentin polyclonal antibody (Product # PA1-10003) at a dilution 1:5,000 as seen in red, and costained using a MAP2C/D monoclonal antibody at a dilution 1:5,000 as seen in green. 1) protein standard (red), 2) rat whole brain lysate, 3) HeLa, 4) SH-SY5Y, 5) HEK293, and 6) NIH-3T3 cell lysates. Vimentin protein is bound showing a single band at ~50 kDa., Full length MAP2A/2B is depicted by multiple bands around 280 kDa, and MAP2C/D isotypes ~70 kDa bands. MAP2 isotypes are seen only in extracts containing neuronal lineage cells.



Vimentin Antibody (PA1-10003)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in Vimentin KO cell line compared to control cell line using Anti-Vimentin Polyclonal Antibody (Product # PA1-10003). {KO}

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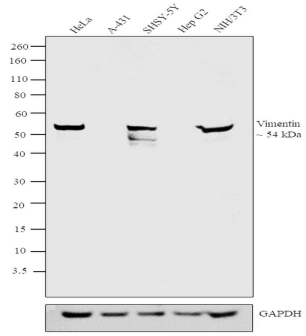
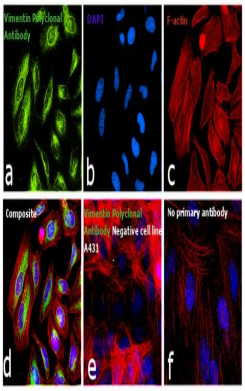


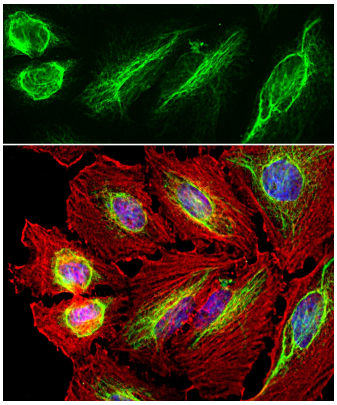
Vimentin Antibody (PA1-10003)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell lines owing to their inherent genetic constitution. Relative expression of Vimentin was observed in all cell lines tested using product (Product # PA1-10003) in western blot. {RE}



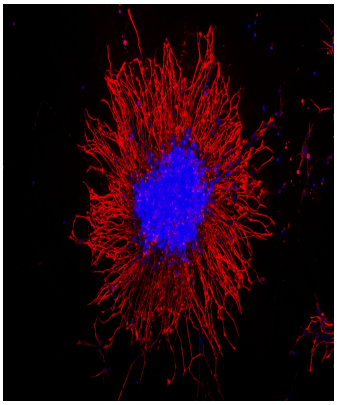
Vimentin Antibody (PA1-10003) in ICC/IF

Immunofluorescence analysis of Vimentin was performed using 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with Vimentin Chicken Polyclonal Antibody (Product # PA1-10003) at 1:200 dilution in 0.1% BSA and incubated overnight at 4 degree Celsius and then labeled with Goat anti-Chicken IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A-11039) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cytoplasmic, cytoskeletal and nuclear localization. Panel e represents negative control, A-431 cells. Panel f represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



Vimentin Antibody (PA1-10003) in ICC/IF

Immunofluorescent analysis of Vimentin in HeLa cell culture stained a Vimentin polyclonal antibody (Product # PA1-10003) at a dilution of 1:10,000 as seen in green, and costained with an Actin monoclonal antibody at a dilution of 1:500 in red, and with DAPI staining the nuclear DNA in blue. 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.



Vimentin Antibody (PA1-10003) in ICC/IF

Immunofluorescent analysis of Vimentin in hN2 cell culture. After 4 days islands of hN2 cells form. Samples were then fixed and stained with a Vimentin polyclonal antibody (Product # PA1-10003) as seen in red, and with DAPI staining the nuclear DNA in blue. The final image was merged from several images taken with a 10X objective lens.

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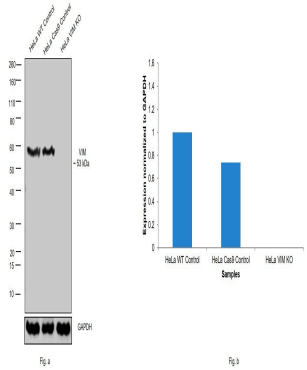

Vimentin Antibody (PA1-10003) in WB

Knockout of Vimentin was achieved by CRISPR-Cas9 genome editing using LentiArray™ Lentiviral sgRNA (Product # A32042) and LentiArray Cas9 Lentivirus (Product # A32064). Western blot analysis of Vimentin was performed by loading 30 µg of HeLa wild type (Lane 1), HeLa CAS9 (Lane 2) and HeLa Vimentin KO (Lane 3) whole cell extracts. The samples were electrophoresed using Novex® NuPAGE® 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Anti-Vimentin Polyclonal Antibody (Product # PA1-10003) using 1:2,000 dilution and Goat anti-Chicken IgY (H+L) Secondary Antibody, HRP (Product # A16054, 1:4,000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005). Loss of signal upon CRISPR mediated knockout (KO) using the LentiArray™ CRISPR product line confirms that antibody is specific to Vimentin.

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PubMed References For Vimentin Polyclonal Antibody

1 Immunocytochemistry References

Species / Dilution	Summary
Rat / 1:1000	PA1-10003 was used in Immunocytochemistry-immunofluorescence to assess the modulatory roles of astrocytes during epileptic-like conditions and in compensating drug-elicited hyperactivity. International journal of molecular sciences (Nov 2021; 22:) **"Astrocytes Exhibit a Protective Role in Neuronal Firing Patterns under Chemically Induced Seizures in Neuron-Astrocyte Co-Cultures."** Author(s):Ahtiainen A,Genocchi B,Tanskanen JMA,Barros MT,Hyttinen JAK,Lenk K PubMed Article URL:http://dx.doi.org/10.3390/ijms222312770

1 Miscellaneous PubMed References

Species / Dilution	Summary
Human / Not Cited	PA1-10003 was used in In vivo experiments to study static and perfused models of human myocardial-microvascular interaction using a functionally vascularized in vitro model of human myocardium with widespread potential applications in basic and translational research. Cell reports methods (Sep 2022; 2:) **"Functional microvascularization of human myocardium in vitro."** Author(s):King O,Cruz-Moreira D,Sayed A,Kermani F,Kit-Anan W,Sunyovszki I,Wang BX,Downing B,Fourre J,Hachim D,Randi AM,Stevens MM,Rasponi M,Terracciano CM PubMed Article URL:http://dx.doi.org/10.1016/j.crmeth.2022.100280

3 Immunohistochemistry References

Species / Dilution	Summary
Human / 1:1000	PA1-10003 was used in immunohistochemistry to report on the presence of interstitial Cajal-like cells in the human thoracic duct Cells, tissues, organs (Sep 2013; 197: 145) **"Identification of interstitial Cajal-like cells in the human thoracic duct."** Author(s):Briggs Boedtkjer D,Rumessen J,Baandrup U,Skov Mikkelsen M,Telinius N,Pilegaard H,Aalkjaer C,Hjortdal V PubMed Article URL:http://dx.doi.org/10.1159/000342437
Rat / 1:5000	PA1-10003 was used in Immunohistochemistry to determine how the cardiac fibroblasts secretome changes with matrix stiffness and biochemical cues and how this affects cardiac myocytes via paracrine signaling. Journal of the American Heart Association (Oct 2020; 9:) **"Defining the Cardiac Fibroblast Secretome in a Fibrotic Microenvironment."** Author(s):Ceccato TL,Starbuck RB,Hall JK,Walker CJ,Brown TE,Killgore JP,Anseth KS,Leinwand LA PubMed Article URL:http://dx.doi.org/10.1161/JAHA.120.017025
Rat / 1:2000	PA1-10003 was used in Immunohistochemistry-immunofluorescence to demonstrate the approach of LMS to understand load-triggered cardiac inflammation and, thus, identify potential translationally important therapeutic targets. Basic research in cardiology (Nov 2022; 117:) **"Chemical and mechanical activation of resident cardiac macrophages in the living myocardial slice ex vivo model."** Author(s):Waleczek FJG,Sansonetti M,Xiao K,Jung M,Mitzka S,Dendorfer A,Weber N,Perbellini F,Thum T PubMed Article URL:http://dx.doi.org/10.1007/s00395-022-00971-2

1 Western Blot References

Species / Dilution	Summary
Human / Not Cited	PA1-10003 was used in Western Blotting to show that enhancement in FDPS level is observed in glioma tissues and associate with poor prognosis, contributed to tumour growth. Journal of cellular and molecular medicine (Aug 2020; 24: 9055) **"FDPS promotes glioma growth and macrophage recruitment by regulating CCL20 via Wnt/-catenin signalling pathway."** Author(s):Chen Z,Chen G,Zhao H PubMed Article URL:http://dx.doi.org/10.1111/jcmm.15542

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