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PGP9.5 Polyclonal Antibody

Catalog Number **PA1-10011**

Product data sheet

Details	
Size	100 µL
Host/Isotope	Chicken / IgY
Class	Polyclonal
Type	Antibody
Immunogen	Recombinant full length human UCHL1
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, Horse, Human, Mouse, Pig, Rat
Published species	Cynomolgus monkey, Rat, Human, Not Applicable

Tested Applications	Dilution *
Western Blot (WB)	1:2,000-1:5,000
Immunocytochemistry (ICC/IF)	1:500-1:1,000

Published Applications	
Immunohistochemistry (IHC)	See 2 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

PA1-10011 was made against full length recombinant human UCHL1 expressed in and purified from E. coli and can be used to identify neurons and their processes in culture or in sections. The antibody works cleanly on appropriate lysates of cell and tissues. |Stable at 4°C.

Background/Target Information

PGP9.5 (Protein gene product 9.5, UCH-L1, PARK5) is a neuron specific protein, structurally and immunologically distinct from neuron specific enolase. PGP9.5 has a molecular weight of 27 kDa and was first identified by high resolution two dimensional PAGE. PGP9.5 is a member of ubiquitin carboxyl-terminal hydrolase family 1 (peptidase family C12) with a ubiquitin carboxyl-terminal hydrolase domain. PGP9.5 is well known for having ubiquitin hydrolase and ligase activities that hydrolyzes small C-terminal adducts of ubiquitin to generate ubiquitin monomers. PGP9.5 is present in neurons and nerve fibers at all levels of the central and peripheral nervous system, in neuroendocrine cells, in segments of the renal tubules, in spermatogonia and Leydig cells of the testis, in ova and in some cells of both the pregnant and non-pregnant corpus luteum. Over expression of PGP9.5 leads to non-small cell lung cancer while decreased expression leads to Huntington disease and Alzheimer disease. Since PGP9.5 is present in cellular inclusions, it can be a useful as a neuronal marker and in the studies of neurodegenerative disorders such as with Parkinson disease.

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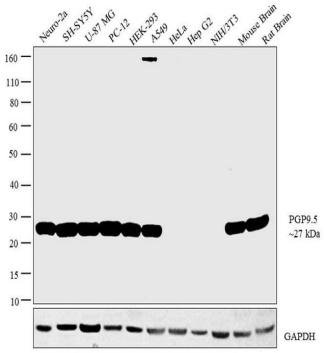
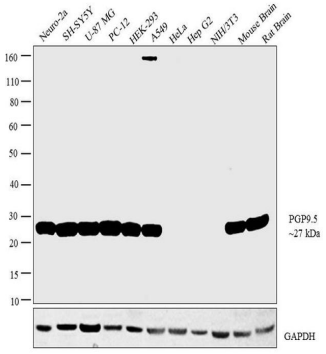
Product Images For PGP9.5 Polyclonal Antibody



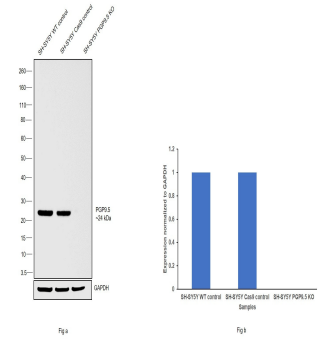
PGP9.5 Antibody (PA1-10011)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. Expression of PGP9.5 was observed in all the cell lines and tissues tested except for HeLa, HepG2 and NIH/3T3 using PGP9.5 Chicken Polyclonal Antibody (Product # PA1-10011) in Western Blot. {RE}



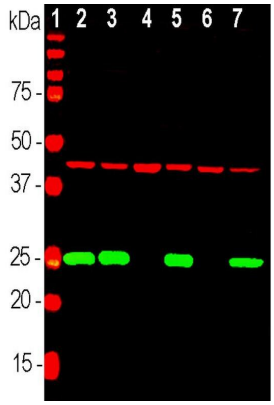
PGP9.5 Antibody (PA1-10011) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of Neuro-2a (Lane 1), SH-SY5Y (Lane 2), U-87 MG (Lane 3), PC-12 (Lane 4), HEK-293 (Lane 5), A549 (Lane 6), HeLa (Lane 7), Hep G2 (Lane 8), NIH/3T3 (Lane 9), tissue extracts of Mouse Brain (Lane 10) and Rat Brain (Lane 11). The blot was probed with Anti-PGP9.5 Polyclonal Antibody (Product # PA1-10011, 1:10,000 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 27 kDa band corresponding to PGP9.5 was detected across the cell lines and tissues tested except for Hela, HepG2 and NIH/3T3 which is reported to be negative for PGP9.5 expression.



PGP9.5 Antibody (PA1-10011)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein in PGP9.5/UCHL1 (KO) cell line compared to control cell line using Anti-PGP9.5/UCHL1 Polyclonal Antibody (Product # PA1-10011). {KO}



PGP9.5 Antibody (PA1-10011) in WB

Western blot analysis of PGP9.5 in tissue and cell lysates using a PGP9.5 polyclonal antibody (Product # PA1-10011) at a dilution of 1:2,000 as seen in green, and using an Actin monoclonal antibody at a dilution of 1:1,000 as seen in red. 1) protein standard, 2) rat brain, 3) mouse brain, 4) NIH-3T3, 5) HEK293, 6) HeLa and 7) SH-SY5Y cells. The single band at 24 kDa mark corresponds to PGP9.5 protein which is detectable in CNS extracts and lysates of cells with neuronal properties but not in lysates of HeLa, NIH-3T3 and other non-neuronal cells. Actin is detected with apparent molecular weight of 42 kDa and provides an excellent loading control.

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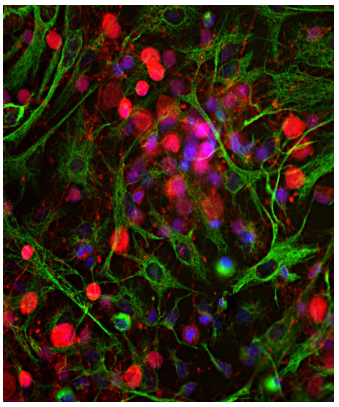
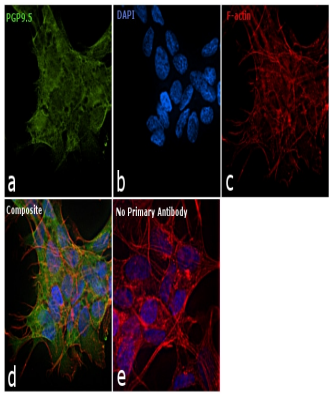


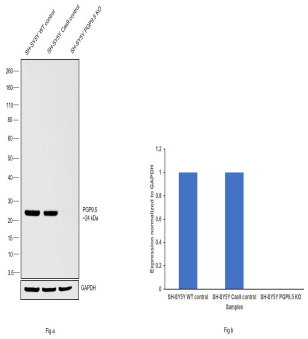
PGP9.5 Antibody (PA1-10011) in ICC/IF

Immunofluorescent analysis of PGP9.5 in cortical neuron-glial cell culture. The culture was prepared from an E20 rat and stained using a PGP9.5 polyclonal antibody (Product # PA1-10011) at a dilution of 1:500 as seen in red, and costained using a Vimentin monoclonal antibody at a dilution 1:2,000 as seen in green, and with DAPI staining the nuclear DNA in blue. The PGP9.5 antibody produces strong staining of the cell body and dendrites in neurons. The vimentin antibody stains intermediate filaments in fibroblastic and developing glial cells.



PGP9.5 Antibody (PA1-10011) in ICC/IF

Immunofluorescence analysis of PGP9.5 was performed using 70% confluent log phase SH-SY5Y 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 PGP9.5 Chicken Polyclonal Antibody (Product # PA1-10011) at 1:1000 dilution in 0.1% BSA and incubated overnight at 4 degree and then labeled with Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor 488 conjugate (Product # A28175) 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 and cytoplasmic localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



PGP9.5 Antibody (PA1-10011) in WB

Western blot analysis of PGP9.5/UCHL1 was performed by loading 20 µg of SH-SY5Y wild type (Lane 1), SH-SY5Y Cas9 control (Lane 2), SH-SY5Y PGP9.5/UCHL1 knockout (Lane 3) whole cell extracts. The blot was probed with Anti-PGP9.5/ UCHL1 Polyclonal Antibody (Product # PA1-10011) (1:10000 dilution) and Goat anti-Chicken IgY (H+L) Secondary Antibody, HRP (Product # A16054) (1:4000 dilution). Loss of signal upon CRISPR mediated knockout (KO) confirms that antibody is specific to PGP9.5/UCHL1.

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PubMed References For PGP9.5 Polyclonal Antibody

2 Immunohistochemistry References

Species / Dilution	Summary
Rat / 1:200	PA1-10011 was used in Immunohistochemistry to present a new protocol for isolation and sample collection of rat myenteric plexus cells for in vivo and in vitro studies. Cellular and molecular neurobiology (2023; 43: 315) **"Methods to Study the Myenteric Plexus of Rat Small Intestine."** Author(s):Hecking I,Stegemann LN,Stahlke S,Theis V,Vorgerd M,Matschke V,Theiss C PubMed Article URL:http://dx.doi.org/10.1007/s10571-021-01181-5
Human / 1:500	PA1-10011 was used in Immunohistochemistry-immunofluorescence to provide critical translational evidence that the common marmoset and rhesus macaque ceca are remarkably similar to the human appendix and, thus, that these NHP species are suitable for studying the development of PD linked to -syn and tau pathological changes in the ENS. PloS one (2022; 17:) **"Alpha-synuclein and tau are abundantly expressed in the ENS of the human appendix and monkey cecum."** Author(s):Zinnen AD,Vichich J,Metzger JM,Gambardella JC,Bondarenko V,Simmons HA,Emborg ME PubMed Article URL:http://dx.doi.org/10.1371/journal.pone.0269190

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