abcam

Product datasheet

Anti-PGP9.5 antibody ab27053

KO VALIDATED

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Overview

Product name	Anti-PGP9.5 antibody
Description	Rabbit polyclonal to PGP9.5
Host species	Rabbit
Specificity	From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help.
Tested applications	**Suitable for:** IHC-P, IP, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide corresponding to Human PGP9.5 aa 150 to the C-terminus conjugated to keyhole limpet haemocyanin.
Positive control	ICC/IF: PC12 cells and primary rat neurons/glia, DIV14 cells. WB: Wild type HAP1 whole cell lysate. HEK-293 whole cell lysate. Human, rat and mouse brain whole cell lysate. IHC-P: Human pancreas tissue. IP: Mouse brain tissue lysate.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS

	Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab27053 in the following tested applications.

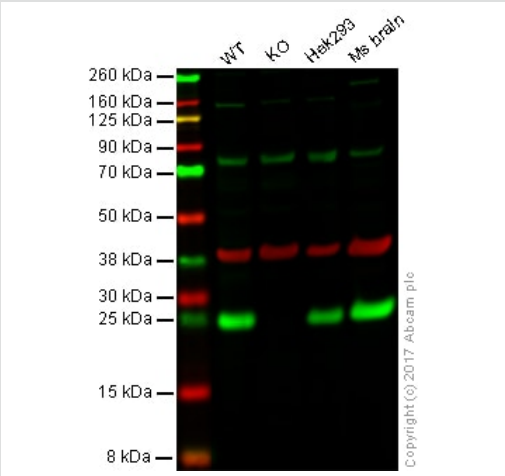
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	★★★☆☆ (3)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IP		Use a concentration of 5 µg/ml.
WB	★★★★☆ (1)	Use a concentration of 1 µg/ml. Detects a band of approximately 25 kDa (predicted molecular weight: 25 kDa). Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented below.
ICC/IF	★★★★☆ (1)	Use a concentration of 1 µg/ml.

Target

Function	Ubiquitin-protein hydrolase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins. This enzyme is a thiol protease that recognizes and hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin. Also binds to free monoubiquitin and may prevent its degradation in lysosomes. The homodimer may have ATP-independent ubiquitin ligase activity.
Tissue specificity	Found in neuronal cell bodies and processes throughout the neocortex (at protein level). Expressed in neurons and cells of the diffuse neuroendocrine system and their tumors. Weakly expressed in ovary. Down-regulated in brains from Parkinson disease and Alzheimer disease patients.
Involvement in disease	Parkinson disease 5 Neurodegeneration with optic atrophy, childhood-onset
Sequence similarities	Belongs to the peptidase C12 family.
Post-translational modifications	O-glycosylated.
Cellular localization	Cytoplasm. Endoplasmic reticulum membrane. About 30% of total UCHL1 is associated with membranes in brain.

Images

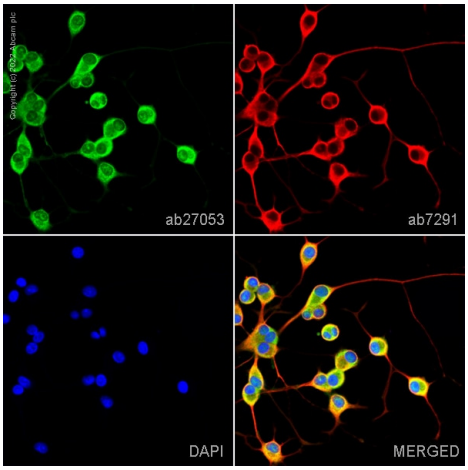


Western blot - Anti-PGP9.5 antibody (ab27053)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: UCHL1 (PGP9.5) knockout HAP1 whole cell lysate (20 µg)
Lane 3: Hek293 whole cell lysate (20 µg)
Lane 4: Ms brain whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab27053 observed at 24 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

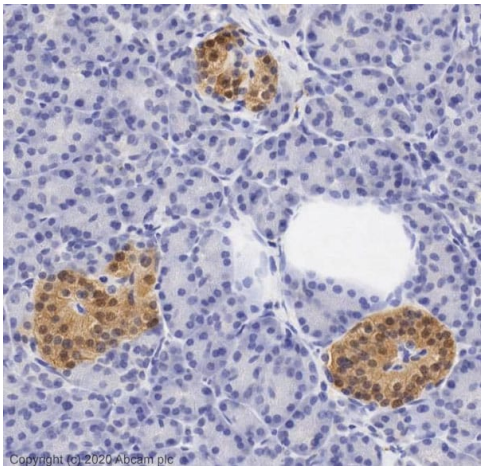
ab27053 was shown to recognize UCHL1 (PGP9.5) in wild type cells as signal was lost at the expected MW in UCHL1 (PGP9.5) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and UCHL1 (PGP9.5) knockout samples were subjected to SDS-PAGE. Ab27053 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-PGP9.5 antibody (ab27053)

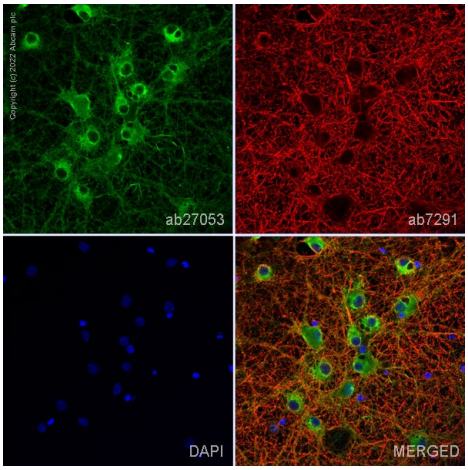
ab27053 staining PGP9.5 in PC12 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab27053 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody (ab27053)

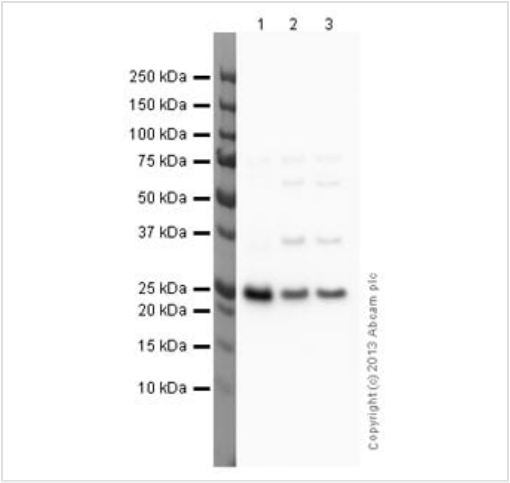
IHC image of Anti-PGP9.5 antibody staining in a section of formalin-fixed paraffin-embedded
normal human pancreas performed on a Leica BOND™ system using the standard protocol. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab27053, 1 µg/mL, for 15 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.



Immunocytochemistry/ Immunofluorescence - Anti-PGP9.5 antibody (ab27053)

ab27053 staining PGP9.5 in primary rat neurons/glia, DIV14 (prepared from E18 rat hippocampal brain area, obtained from Transnetyx Tissue by BrainBits, LLC, cat.no. SDHEP) cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab27053 at 5µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Western blot - Anti-PGP9.5 antibody (ab27053)

All lanes : Anti-PGP9.5 antibody (ab27053) at 1 µg/ml

Lane 1 : Human brain tissue lysate - total protein (**ab29466**)
Lane 2 : Brain (Mouse) Tissue Lysate (**ab27253**)
Lane 3 : Brain (Rat) Tissue Lysate (**ab7942**)

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Developed using the ECL technique.

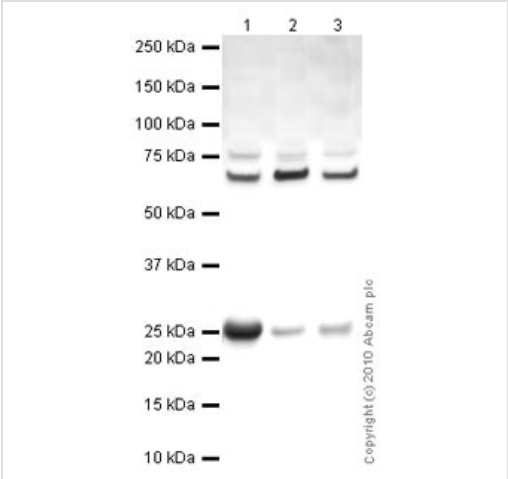
Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 25 kDa
Additional bands at: 37 kDa (possible non-specific binding), 60 kDa (possible non-specific binding), 75 kDa (possible non-specific binding)

Exposure time: 10 seconds

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with abX overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.

Abcam recommends using milk as the blocking agent.



Western blot - Anti-PGP9.5 antibody (ab27053)

All lanes : Anti-PGP9.5 antibody (ab27053) at 1 µg/ml

Lane 1 : Human brain tissue lysate - total protein (**ab29466**)
Lane 2 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate
Lane 3 : Brain (Mouse) Tissue Lysate

Lysates/proteins at 10 µg per lane.

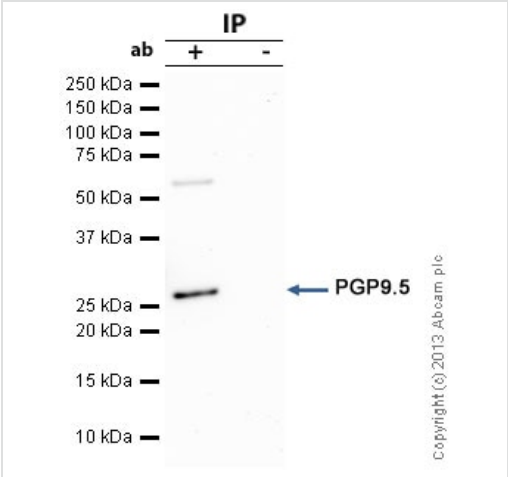
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 25 kDa
Additional bands at: 65 kDa. We are unsure as to the identity of these extra bands.

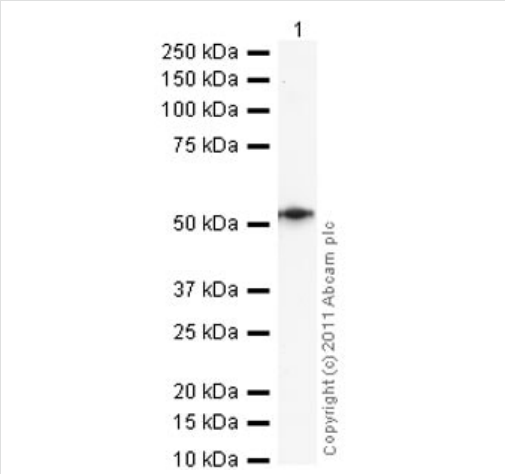
Exposure time: 1 minute



Immunoprecipitation - Anti-PGP9.5 antibody (ab27053)

PGP9.5 was immunoprecipitated using 0.5mg Mouse Brain tissue lysate, 5µg of Rabbit polyclonal to PGP9.5 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).
The antibody was incubated under agitation with Protein G beads for 10min, Mouse Brain tissue lysate lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab27053.
Secondary: Clean-Blot IP Detection Reagent (HRP) at 1/500 dilution.
Band: 26kDa, non specific band - 65kDa: We are unsure as to the

identity of this extra band; PGP9.5



Western blot - Anti-PGP9.5 antibody (ab27053)

Anti-PGP9.5 antibody (ab27053) at 1/250 dilution + Recombinant Human PGP9.5 protein (**ab82628**) at 0.01 µg

Secondary

Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) (**ab65484**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 25 kDa

Exposure time: 3 minutes

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