abcam

Product datasheet

Anti-PGP9.5 antibody ab27053

敲除验证

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概述

产品名称	Anti-PGP9.5抗体
描述	**兔多克隆抗体**to PGP9.5
宿主	Rabbit
经测试应用	**适用于:** IHC-P, IP, WB, IHC-Fr, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide conjugated to KLH derived from within residues 150 to the C-terminus of Human PGP9.5.**参阅**Abcam**的专有抗源政策**(Peptide available as ab27848.)
阳性对照	This antibody gave a positive signal in the following lysates: Mouse Brain Tissue, Mouse Brain Tissue (0 days old), Rat Brain Tissue, Human Brain Tissue, HEK293 and HAP1 Whole Cell

性能

形式	Liquid
存放说明	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.
存储溶液	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS. pH 7.4
纯度	Immunogen affinity purified
克隆	**多克隆**
同种型	IgG

应用

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.

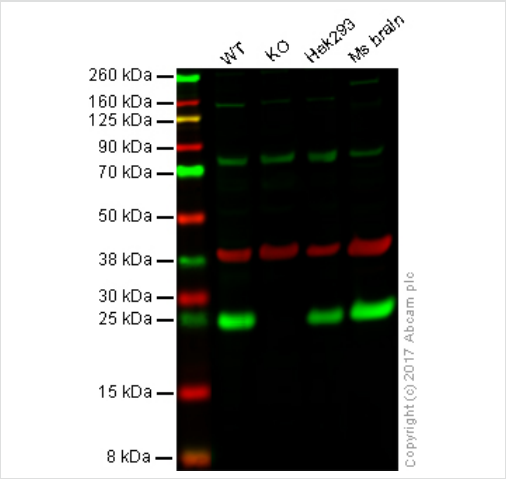
应用	Ab评论	说明

应用	Ab评论	说明
IHC-P	★★★☆☆	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	★★★★☆	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.
IHC-Fr	★★★★★	Use at an assay dependent concentration.
ICC/IF	★★★★☆	Use a concentration of 5 µg/ml.

靶标

功能	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.
组织特异性	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.
疾病相关	Parkinson disease 5 Neurodegeneration with optic atrophy, childhood-onset
序列相似性	Belongs to the peptidase C12 family.
翻译后修饰	O-glycosylated.
细胞定位	Cytoplasm. Endoplasmic reticulum membrane. About 30% of total UCHL1 is associated with membranes in brain.

图片

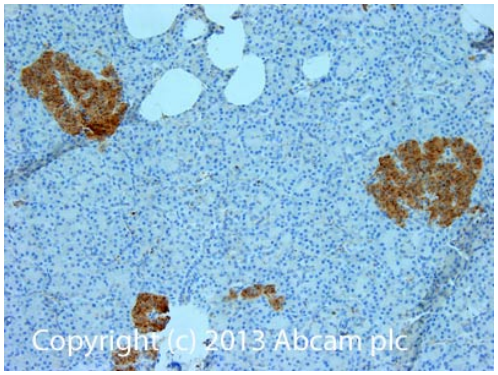


Western blot - Anti-PGP9.5 antibody (ab27053)

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)

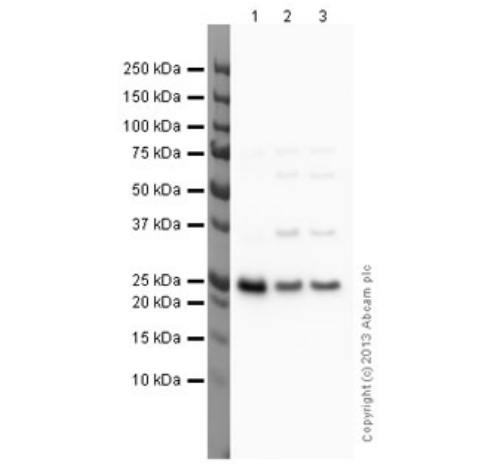
Lanes 1 - 4: Merged signal (red and green). Green - ab27053 observed at 24 kDa. Red - loading control, ab8245, observed at 37 kDa.

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.



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

IHC image of PGP9.5 staining in human pancreas formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab27053, 5µg/ml, for 15 mins 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.



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.

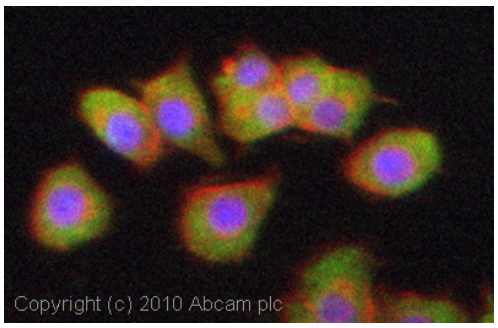
Performed under reducing conditions.

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

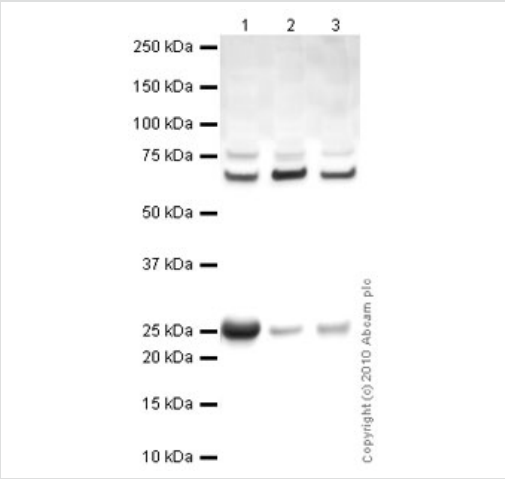
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with abX overnight at 4°C. 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.



Immunocytochemistry/ Immunofluorescence - PGP9.5 antibody - Neuronal Marker (ab27053)

ICC/IF image of ab27053 stained PC12 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab27053, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - PGP9.5 antibody - Neuronal Marker (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.

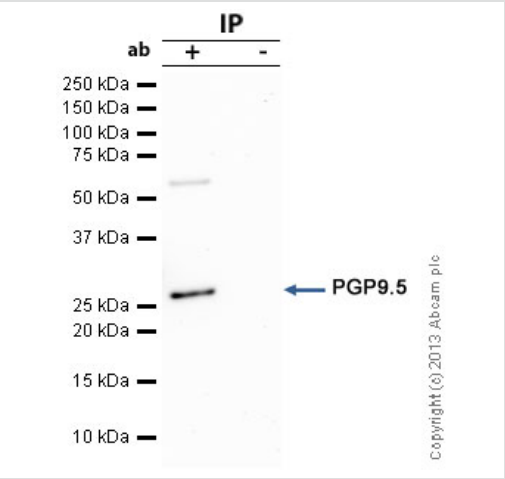
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

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 - Neuronal Marker (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) 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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