abcam

Product datasheet

Anti-PGP9.5 antibody [13C4 / I3C4] ab8189

KO VALIDATED

36 Abreviews 115 References 7 Images

Overview

Product name	Anti-PGP9.5 antibody [13C4 / I3C4]
Description	Mouse monoclonal [13C4 / I3C4] to PGP9.5
Host species	Mouse
Tested applications	**Suitable for:** ICC, IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Sheep, Rabbit, Guinea pig, Dog, Pig, Zebrafish
Immunogen	Full length native protein (purified). This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1 whole cell lysate. Human, mouse and rat brain tissue lysate. Rat cortex tissue lysate. SHSY-5Y whole cell lysate. Human spinal cord tissue lysate. IHC-P: Rat pancreas tissue. ICC: Primary rat neurons/glia, DIV14 cells.
General notes	This antibody labels the neuronal cell bodies and axons in central and peripheral neural system. Small nerve fibers in peripheral tissues, neuroendocrine cells in normal pituitary thyroid, pancreas, and gastrointestinal tract, as well as derived tumors are also stained with this antibody. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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 Some batches contain L-Arginine or BSA as a stabilizing agent. For lot-specific buffer information, please contact our Scientific Support team.
Purity	Protein G purified
Primary antibody notes	This antibody labels the neuronal cell bodies and axons in central and peripheral neural system. Small nerve fibers in peripheral tissues, neuroendocrine cells in normal pituitary thyroid, pancreas, and gastrointestinal tract, as well as derived tumors are also stained with this antibody.
Clonality	Monoclonal
Clone number	13C4 / l3C4
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab8189 in the following tested applications.

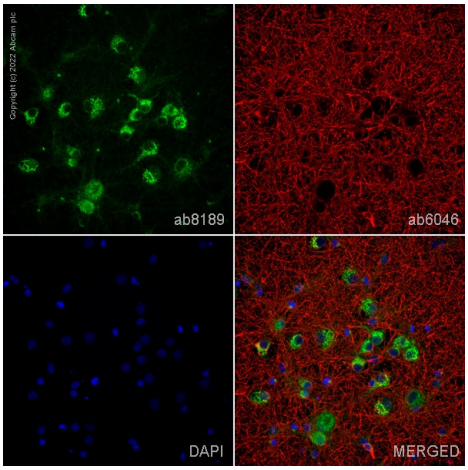
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC		Use a concentration of 5 µg/ml.
IHC-P	★★★★☆ (18)	Use a concentration of 0.5 - 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	★★★★☆ (7)	Use a concentration of 5 µg/ml. Detects a band of approximately 25 kDa (predicted molecular weight: 25 kDa).

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.

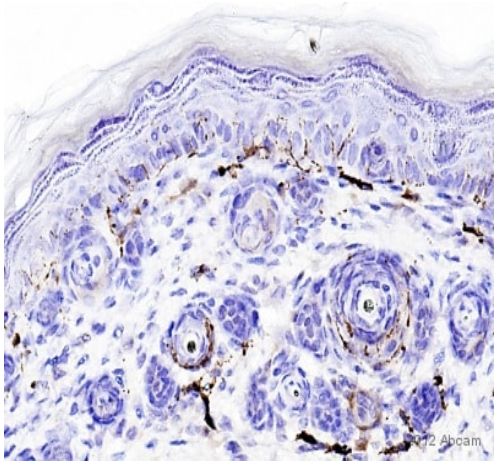
Images



Immunocytochemistry - Anti-PGP9.5 antibody [13C4 / I3C4] (ab8189)

ab8189 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 ab8189 at 5µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150121**, Goat polyclonal Secondary Antibody to Mouse IgM - mu chain (Alexa Fluor® 488) at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

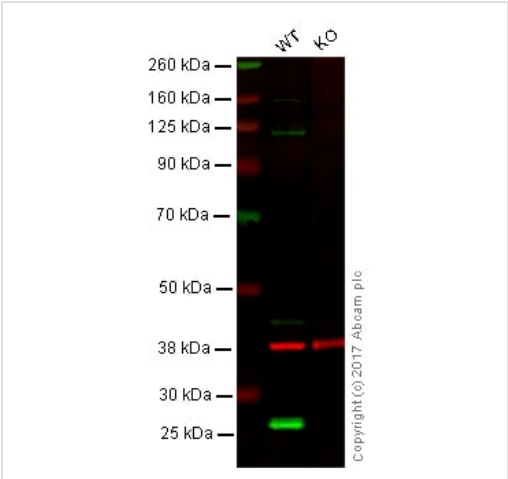


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [13C4 / I3C4] (ab8189)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

IHC-P image of PGP9.5 staining on P5 mouse skin sections using ab8189 (1/1000).

The sections were de-paraffinized and subjected to heat meadiated antigen retrieval using citric acid. The sections were then blocked using 1% BSA for 10 mins at 21°C. ab8189 was then incubated for 16 hours at 21°C. The secondary antibody used was Got polyclonal to anti-mouse IgG conjugated to biotin (1/200).

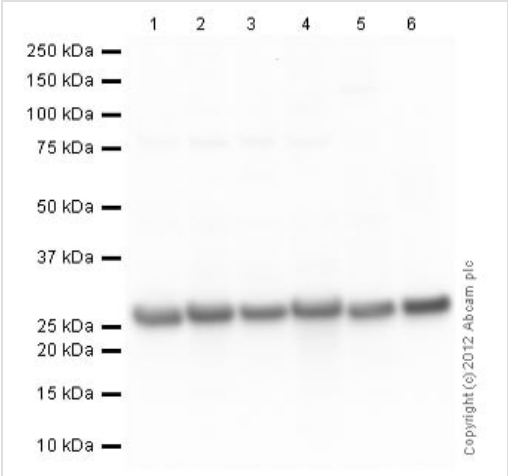


Western blot - Anti-PGP9.5 antibody [13C4 / I3C4] (ab8189)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: PGP9.5 knockout HAP1 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab8189 observed at 25 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab8189 was shown to specifically react with PGP9.5 in wild-type HAP1 cells as signal was lost in PGP9.5 knockout cells. Wild-type and PGP9.5 knockout samples were subjected to SDS-PAGE. ab8189 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 5 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-PGP9.5 antibody [13C4 / I3C4] (ab8189)

All lanes : Anti-PGP9.5 antibody [13C4 / I3C4] (ab8189) at 5 µg/ml

Lane 1 : Human brain tissue lysate - total protein (**ab29466**)
Lane 2 : Brain (Rat) Tissue Lysate
Lane 3 : Brain (Mouse) Tissue Lysate
Lane 4 : Rat Cortex Tissue Lysate
Lane 5 : SHSY-5Y (Human neuroblastoma cell line) Whole Cell Lysate
Lane 6 : Human spinal cord tissue lysate - total protein (**ab29188**)

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution

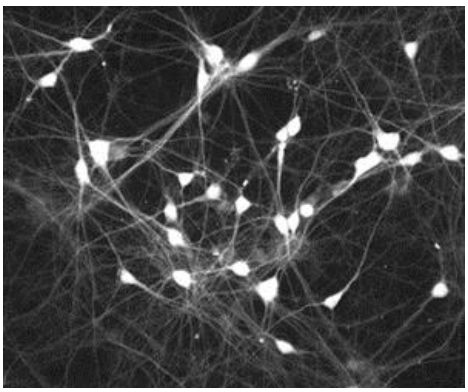
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 1 minute

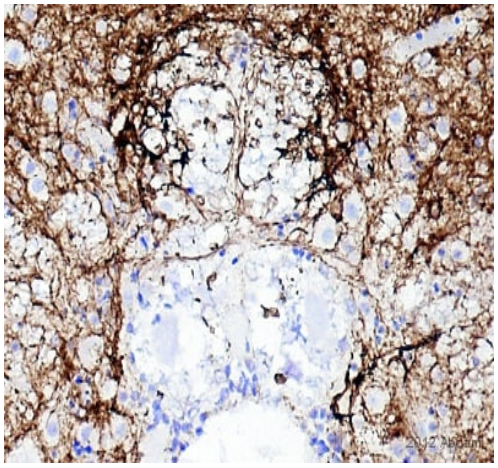
This blot was produced using a 10% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 5% Bovine Serum Albumin before being incubated with ab8189 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution.



Immunocytochemistry - Anti-PGP9.5 antibody [13C4 / I3C4] (ab8189)

Image courtesy of QBMCellScience

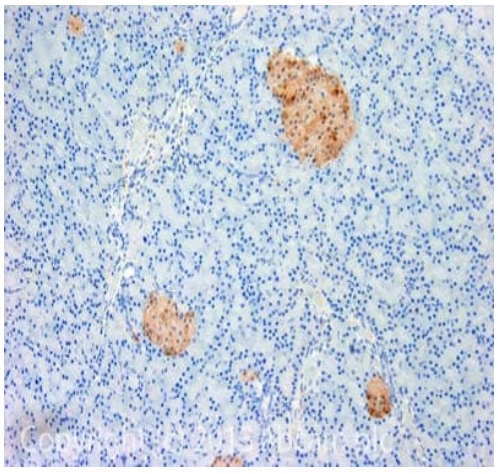
ab8189 (1/20) immunostaining neurons in mouse cortical primary cell culture.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [13C4 / I3C4] (ab8189)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

IHC-P image of PGP9.5 staining on zebrafish brain using ab8189 (1/1000). The sections were subjected to heat mediated antigen retrieval using citric acid. The sections were then blocked using 1% BSA for 10 mins for 21°C. The primary antibody (ab8189) was incubated at a dilution of 1/1000 at 21°C for 16 hours. The secondary antibody used was undiluted goat polyclonal to Mouse IgG conjugated to biotin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [13C4 / I3C4] (ab8189)

IHC image of PGP9.5 staining in rat pancreas formalin-fixed, paraffin-embedded tissue section, performed on a Leica Bond™ 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 ab8189, 0.02µg/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 hematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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