abcam

Product datasheet

Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free ab220823

KO VALIDATED Recombinant RabMAb

4 References 17 Images

Overview

Product name	Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free
Description	Rabbit monoclonal [EPR4118] to PGP9.5 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-Fr, WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Fetal brain, Y79, U87-MG, SH-SY5Y, HAP1, HEK-293, and 293T cell lysates; IHC-P: Human glioma, colon, and hepatocellular carcinoma tissue, Mouse colon and cerebral cortex tissue, Rat Jejunum and cerebral cortex tissue; ICC/IF: Neuro-2a cells; IP: Human fetal brain lysate; Flow Cyt (intra): SH-SY5Y, Neuro2a cells and Y79 cells; IHC-Fr: Mouse cerebrum tissue.
General notes	ab220823 is the carrier-free version of **ab108986**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4118
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab220823 in the following tested applications.

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

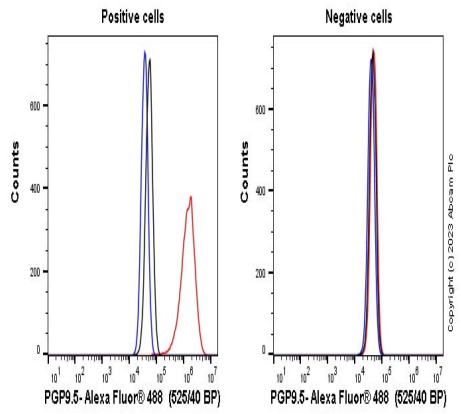
Application	Abreviews	Notes
IHC-Fr		Use at an assay dependent concentration. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
WB		Use at an assay dependent concentration. Detects a band of approximately 25 kDa (predicted molecular weight: 24 kDa).
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

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



Flow Cytometry (Intracellular) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108986**).

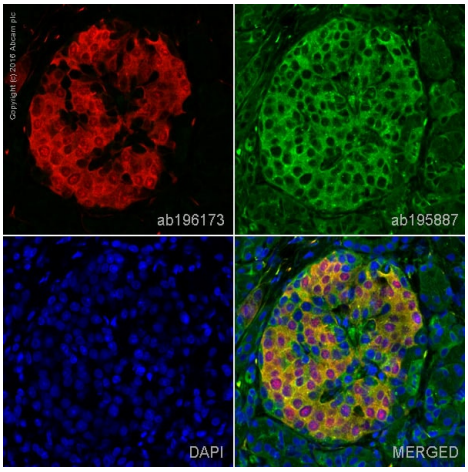
Flow cytometry overlay histogram showing left Neuro2a positive cells and right negative NIH3T3 stained with **ab108986** (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab108986**) (1x 10^6 in 100µl at 0.2µg/ml (1/10500)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Neuro2a Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

Clone EPR4118 (ab220823) has been successfully conjugated by Abcam. This image was generated using Anti-PGP9.5 antibody [EPR4118] (Alexa Fluor® 647). Please refer to **ab196173** for protocol details.

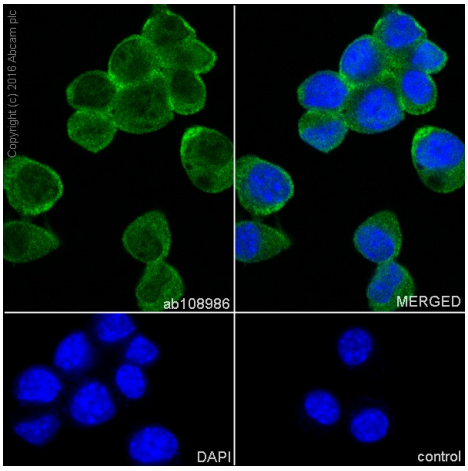
IHC image of PGP9.5 staining in a section of formalin-fixed paraffin-embedded normal human pancreas*.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) in a Dako Pascal pressure cooker using the standard factory-set regime. Non-specific protein-protein interactions were then blocked in TBS containing 0.025% (v/v) Triton X-100, 0.3M (w/v) glycine and 1% (w/v) BSA for 1h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.025% (v/v) Triton X-100 and 1% (w/v) BSA with **ab196173** at 1/100 (shown in red) and counterstained using **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

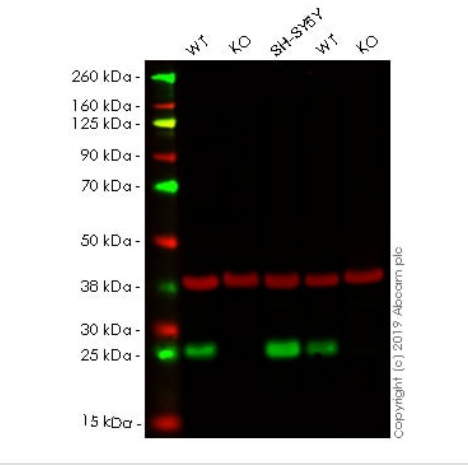


Immunocytochemistry/ Immunofluorescence - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

This ICC/IF data was generated using the same anti-PGP9.5 antibody clone, EPR4118, in a different buffer formulation (cat# **ab108986**).

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Neuro-2a (Mouse neuroblastoma cell line) cells labeling PGP9.5 with **ab108986** at 1/500 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on Neuro-2a cell line. The nuclear counter stain is DAPI (blue).

The negative control is PBS only.



Western blot - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

All lanes : Anti-PGP9.5 antibody [EPR4118] - Neuronal Marker (**ab108986**) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : UCHL1 knockout HAP1 cell lysate
Lane 3 : SH-SY5Y cell lysate
Lane 4 : Wild-type HEK-293T cell lysate
Lane 5 : UCHL1 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

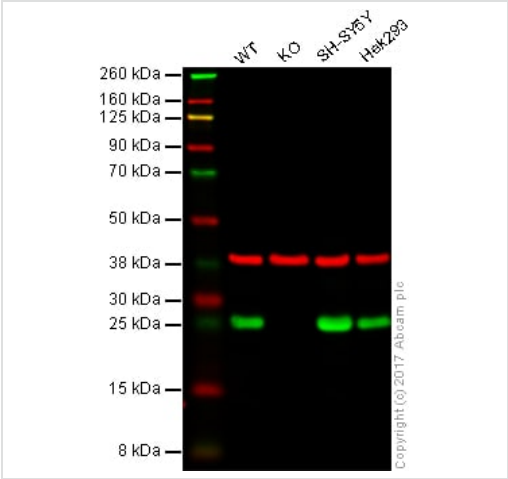
Predicted band size: 24 kDa
Observed band size: 25 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab108986**).

Lanes 1-4: Merged signal (red and green). Green - **ab108986** observed at 25 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab108986 was shown to react with PGP9.5 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout sample **ab263773** was used. Wild-type and PGP9.5 knockout samples were subjected to SDS-PAGE. **ab108986** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated

overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

All lanes : Anti-PGP9.5 antibody [EPR4118] - Neuronal Marker (**ab108986**) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : UCHL1 knockout HAP1 whole cell lysate
Lane 3 : SH-SY5Y whole cell lysate
Lane 4 : HEK293 whole cell lysate

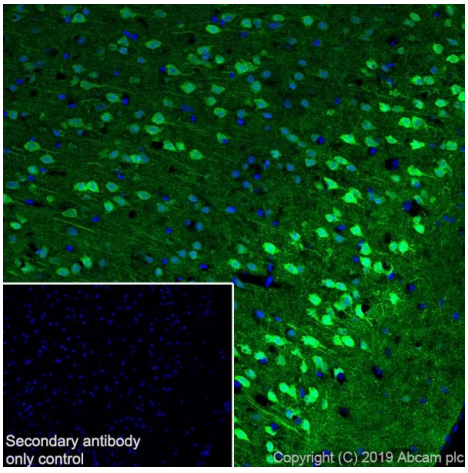
Lysates/proteins at 20 µg per lane.

Predicted band size: 24 kDa

This WB data was generated using the same anti-PGP9.5 antibody clone, EPR4118, in a different buffer formulation (cat# **ab108986**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab108986** observed at 24 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

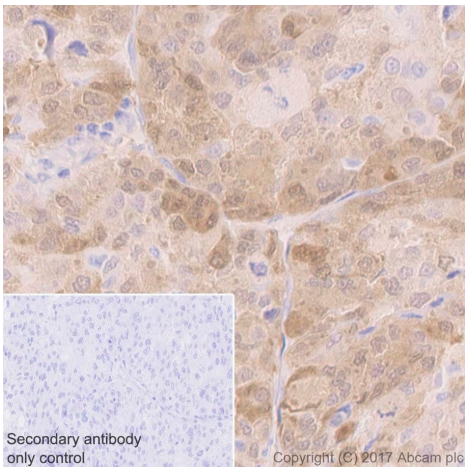
Ab108986 was shown to specifically react with UCHL1 (KO) in wild-type cells as signal was lost in UCHL1 (KO) knockout HAP1 cells. Wild-type and UCHL1 (KO) knockout samples were subjected to SDS-PAGE. Ab108986 and **ab8245** (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution 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 (Frozen sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

Immunohistochemistry (Frozen sections) analysis of mouse cerebrum tissue sections labeling PGP9.5 with Purified **ab108986** at 1/250 (0.5 µg/ml).Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. DAPI was used as a counterstain.

This data was generated using the same anti-PGP9.5 antibody clone, EPR4118, in a different buffer formulation (cat# **ab108986**).

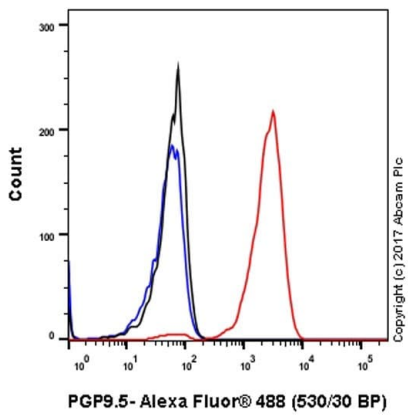


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma tissue labeling PGP9.5 with **ab108986**, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on human hepatocellular carcinoma. The section was incubated with **ab229902** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

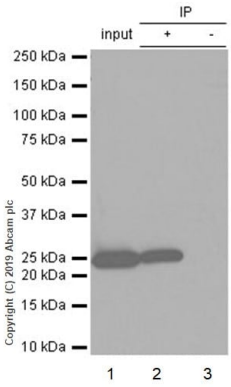
This data was generated using the same anti-PGP9.5 antibody clone, EPR4118, in a different buffer formulation (cat# **ab108986**).



Flow Cytometry (Intracellular) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Y79 (Human retinoblastoma retinoblastoma) cells labelling PGP9.5 with **ab108986** at 1/20 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (black)and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor®488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

This data was generated using the same anti-PGP9.5 antibody clone, EPR4118, in a different buffer formulation (cat **ab108986**).



Immunoprecipitation - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

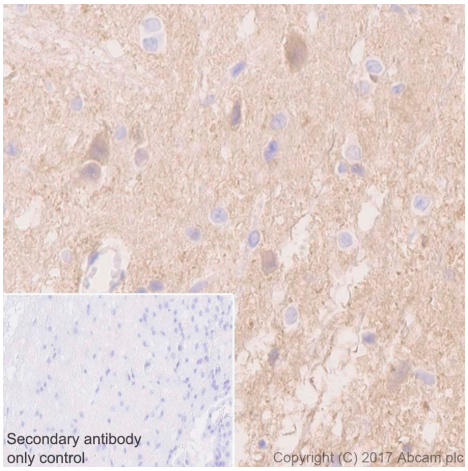
PGP9.5 was immunoprecipitated from 0.35 mg Human fetal brain lysate with **ab108986** at 1/20 dilution (0.5µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab108986** 1/500 dilution (0.17 µg/ml). VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used as the secondary antibody at 1/1000 dilution.

Lane 1: Human fetal brain lysate 10µg
Lane 2: **ab108986** IP in Human fetal brain lysate
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab108986** in Human fetal brain lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

This data was generated using the same anti-PGP9.5 antibody clone, EPR4118, in a different buffer formulation (cat# **ab108986**).

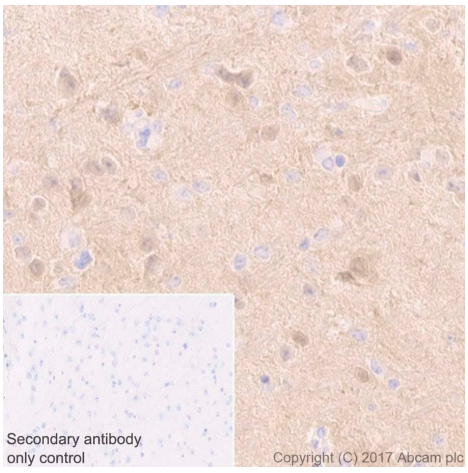


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

Immunohistochemical analysis of paraffin-embedded rat cerebral cortex tissue labeling PGP9.5 with **ab108986**, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on rat cerebral cortex.The section was incubated with **ab229902** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

This data was generated using the same anti-PGP9.5 antibody clone, EPR4118, in a different buffer formulation (cat# **ab108986**).

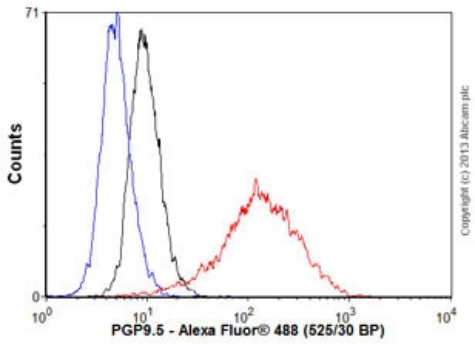


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

Immunohistochemical analysis of paraffin-embedded mouse cerebral cortex tissue labeling PGP9.5 with **ab108986**, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Positive staining on mouse cerebral cortex. The section was incubated with **ab229902** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

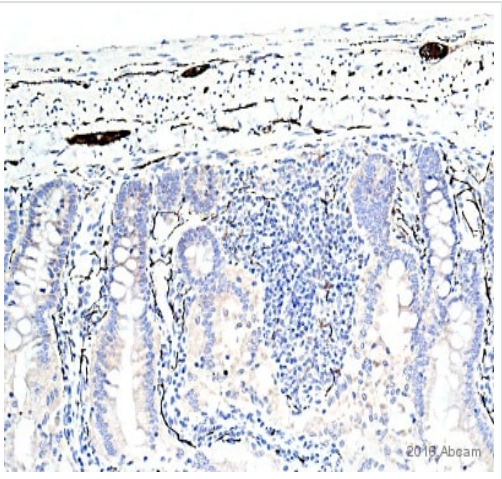
This data was generated using the same anti-PGP9.5 antibody clone, EPR4118, in a different buffer formulation (cat# **ab108986**).



Flow Cytometry (Intracellular) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

Overlay histogram showing SH-SY5Y cells stained with **ab108986** (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (**ab108986**, 1/10000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in SH-SY5Y cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108986**).



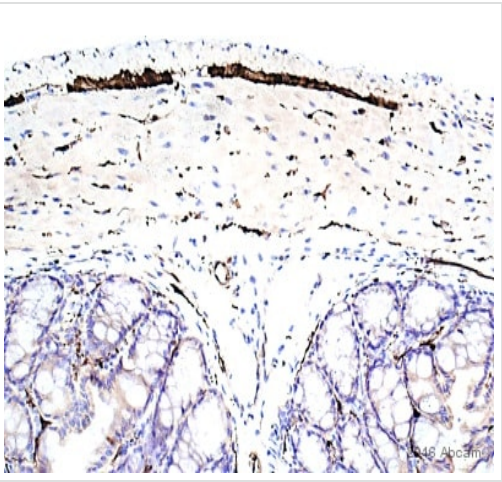
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

This image is courtesy of an Abreview submitted by Carl Hobbs

Immunohistochemical analysis of rat Jejunum tissue sections labeling PGP9.5 with **ab108986** at a dulution of 1/1000. Sections were fixed with Formaldehyde. A Biotin conjugated Goat Anti-Rabbit IgG at 1/300 was used as the secondary antibody. Antigen retrieval was heat mediated using citric acid.

All nerve components of enteric plexuses appear to be very well demonstrated, particularly the fine fibres of the lamina propria and the muscularis mucosa.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108986**).



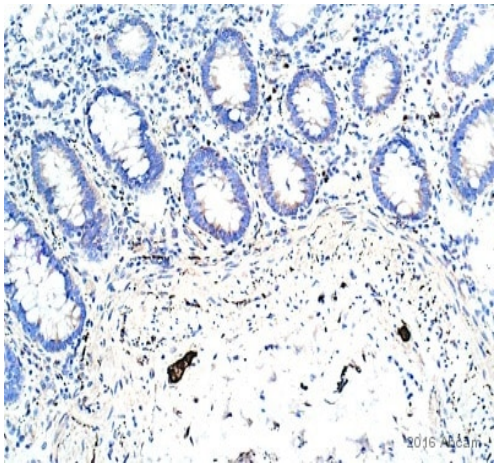
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

This image is courtesy of an Abreview submitted by Carl Hobbs

Immunohistochemical analysis of mouse colon tissue sections labeling PGP9.5 with **ab108986** at a dulution of 1/1500. Sections were fixed with Formaldehyde. A Biotin conjugated Goat Anti-Rabbit IgG at 1/300 was used as the secondary antibody. Antigen retrieval was heat mediated using citric acid.

All nerve cell/fibre components of enteric plexuses are demonstrated very well.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108986**).

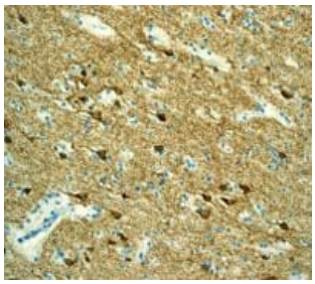


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

This image is courtesy of an Abreview submitted by Carl Hobbs.

ab108986 staining PGP9.5 in human colon tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 2% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in citric acid. Samples were incubated with the primary antibody (1/500 in TBS/BSA/azide) for 16 hours at 21°C. A Biotin-conjugated goat anti-rabbit IgG polyclonal (1/300) was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108986**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

Immunohistochemical staining of PGP9.5 in paraffin embedded Human glioma tissue, using **ab108986** at a 1/250 dilution.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108986**).

Anti-PGP9.5 antibody [EPR4118] - BSA and Azide free (ab220823)

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