# abcam

### Product datasheet

# Anti-PGP9.5 antibody [346CT2.5.1] ab86808



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Overview

Product name Anti-PGP9.5 antibody [346CT2.5.1]

**Description** Mouse monoclonal [346CT2.5.1] to PGP9.5

Host species Mouse

Tested applications Suitable for: ICC/IF, Flow Cyt (Intra), WB

Species reactivity Reacts with: Rat, Human

Predicted to work with: Mouse, Goat, Horse, Cat, Dog, Pig

**Immunogen** Recombinant full length protein corresponding to Human PGP9.5 aa 1 to the C-terminus.

**Positive control**This antibody gave a positive signal in the following lysates: Human Brain Tissue; Rat Brain

Tissue; Rat Brain Cortex Tissue; PC12 Whole Cell; Y79 Whole Cell; U-87MG Whole Cell; SHSY-

5Y Whole Cell and HAP1 Wole cells.

**General notes**This monoclonal antibody to PGP9.5 has been knockout validated in Western blot. The expected

band for PGP9.5 was observed in wild type cells and the band was not seen in knockout cells.

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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.2

Preservative: 0.02% Sodium azide

Constituent: PBS

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**Purity** Protein G purified

ClonalityMonoclonalClone number346CT2.5.1

Myeloma Sp2/0 lsotype lgG1

# **Applications**

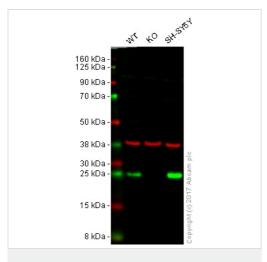
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab86808 in the following tested applications.

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

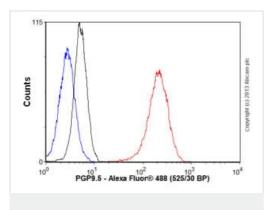
Application	Abreviews	Notes
ICC/IF	<b>★★★★</b> <u>(1)</u>	Use at an assay dependent concentration.
Flow Cyt (Intra)		Use 0.01-0.1µg for 10 <sup>6</sup> cells.  ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 25 kDa (predicted molecular weight: 25 kDa). Abcam recommends blocking with 3% Milk.

larget		
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 [346CT2.5.1] (ab86808)



Flow Cytometry (Intracellular) - Anti-PGP9.5 antibody [346CT2.5.1] (ab86808)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

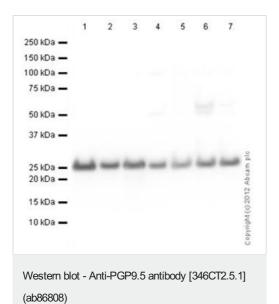
Lane 2: UCHL1 (KO) knockout HAP1 whole cell lysate (20 µg)

Lane 3: SH-SY5Y whole cell lysate (20 µg)

**Lanes 1 - 3:** Merged signal (red and green). Green - ab86808 observed at 24 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

Ab86808 was shown to specifically react with PGP9.5/UCHL1 in wild-type cells as signal was lost in PGP9/UCHL1 knockout HAP1 cells. Wild-type and UCHL1 (KO) knockout samples were subjected to SDS-PAGE. Ab86808 and <a href="mailto:ab181602">ab181602</a> (Rabbit anti GAPDH loading control) were incubated overnight at 4°C at 1 ug/ml and 1/2000 dilution respectively. Blots were developed with Goat anti-Mouse lgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216772">ab216772</a> and Goat anti-Rabbit lgG H&L (IRDye® 680RD) preabsorbed <a href="mailto:ab216777">ab216777</a> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

Overlay histogram showing SH-SH5Y cells stained with ab86808 (red line). The cells were fixed with 80% methanol (5 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 (ab86808, 0.1µg/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse lgG (H&L) (ab150113) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG1 [ICIGG1] (ab91353, 1µg/1x10<sup>6</sup> 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 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



All lanes : Anti-PGP9.5 antibody [346CT2.5.1] (ab86808) at 1  $\mu$ g/ml

Lane 1: Human brain tissue lysate - total protein (ab29466)

Lane 2: Brain (Rat) Tissue Lysate

Lane 3: Rat Cortex Tissue Lysate

Lane 4: PC12 (Rat adrenal pheochromocytoma cell line) Whole

Cell Lysate

Lane 5 : Y79 (Human retinoblastoma cell line) Whole Cell LysateLane 6 : U-87 MG (Human glioblastoma astrocytoma) Whole Cell

Lysate

Lane 7: SHSY-5Y (Human neuroblastoma cell line) Whole Cell

Lysate

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

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