Anti-PGP9.5 Antibody [PSH13-92]

HA723585

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC, IF-Tissue
Molecular Wt:	Predicted band size: 25 kDa
Clone number:	PSH13-92

Description: Ubiquitin carboxy-terminal hydrolase L1 (EC 3.1.2.15, ubiquitin C-terminal hydrolase, UCH-L1) is a deubiquitinating enzyme. UCH-L1 is a member of a gene family whose products hydrolyze small C-terminal adducts of ubiquitin to generate the ubiquitin monomer. Expression of UCH-L1 is highly specific to neurons and to cells of the diffuse neuroendocrine system and their tumors. It is abundantly present in all neurons (accounts for 1-2% of total brain protein), expressed specifically in neurons and testis/ovary. The catalytic triad of UCH-L1 contains a cysteine at position 90, an aspartate at position 176, and a histidine at position 161 that are responsible for its hydrolase activity.

Immunogen: Recombinant protein within human PGP9.5 aa 1-223.

Positive control: A549 cell lysate, LNCaP cell lysate, DU145 cell lysate, SH-SY5Y cell lysate, Neuro-2a cell lysate, C6 cell lysate, A549, Neuro-2a, C6, human appendix tissue, human brain tissue, human pancreas tissue, rat pancreas tissue.

Subcellular location: Cytoplasm, Endoplasmic reticulum membrane.

Database links: SwissProt: P09936 Human | Q9R0P9 Mouse | Q00981 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:2,000
IF-Cell	1:50-1:500
FC	1:10,000
IF-Tissue	1:500

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

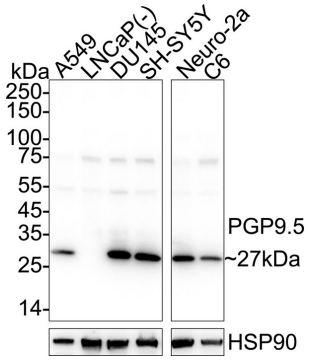

Fig1: Western blot analysis of PGP9.5 on different lysates with Rabbit anti-PGP9.5 antibody (HA723585) at 1/5,000 dilution.

Lane 1: A549 cell lysate
Lane 2: LNCaP cell lysate (negative)
Lane 3: DU145 cell lysate
Lane 4: SH-SY5Y cell lysate
Lane 5: Neuro-2a cell lysate
Lane 6: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 25 kDa
Observed band size: 27 kDa

Exposure time: 2 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA723585) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

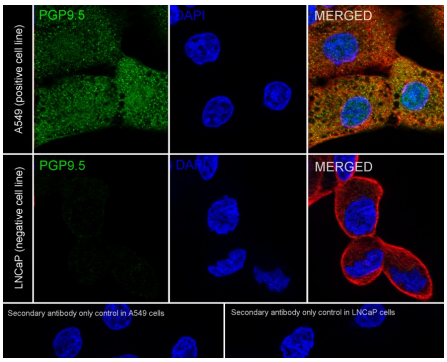

Fig2: Immunocytochemistry analysis of A549 (positive) and LNCaP (negative) labeling PGP9.5 with Rabbit anti-PGP9.5 antibody (HA723585) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-PGP9.5 antibody (HA723585) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

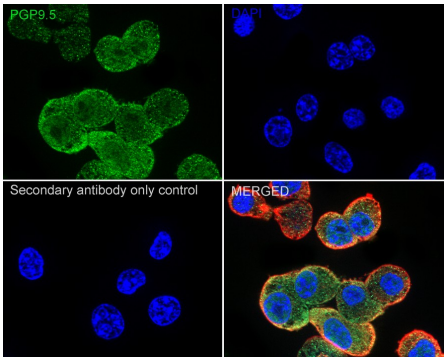

Fig3: Immunocytochemistry analysis of Neuro-2a cells labeling PGP9.5 with Rabbit anti-PGP9.5 antibody (HA723585) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-PGP9.5 antibody (HA723585) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

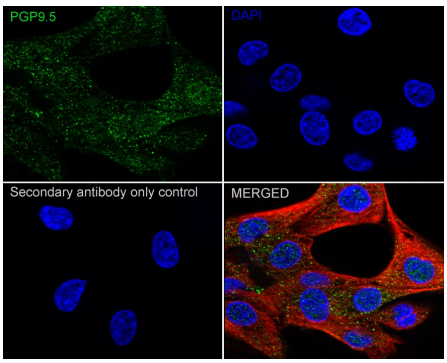

Fig4: Immunocytochemistry analysis of C6 cells labeling PGP9.5 with Rabbit anti-PGP9.5 antibody (HA723585) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-PGP9.5 antibody (HA723585) at 1/50 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

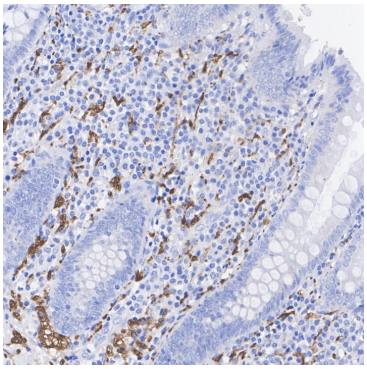

Fig5: Immunohistochemical analysis of paraffin-embedded human appendix tissue with Rabbit anti-PGP9.5 antibody (HA723585) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723585) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

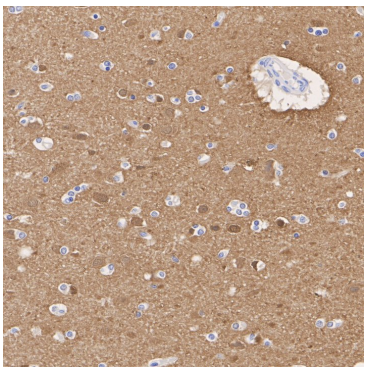

Fig6: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-PGP9.5 antibody (HA723585) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723585) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

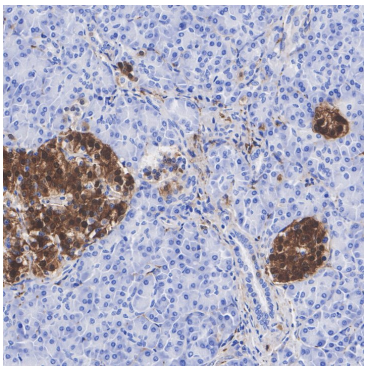

Fig7: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-PGP9.5 antibody (HA723585) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723585) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

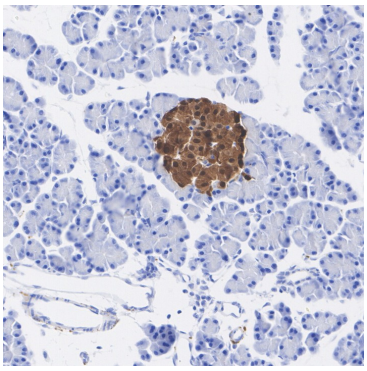

Fig8: Immunohistochemical analysis of paraffin-embedded rat pancreas tissue with Rabbit anti-PGP9.5 antibody (HA723585) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA723585) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

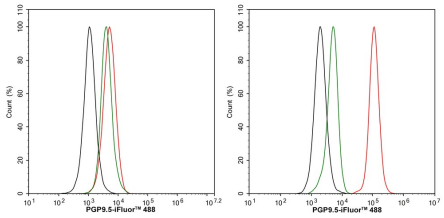

Fig9: Flow cytometric analysis of LNCaP (left, negative) and A549 (right, positive) cells labeling PGP9.5.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723585, 1/10,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

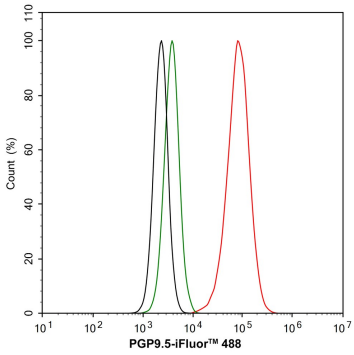

Fig10: Flow cytometric analysis of Neuro-2a cells labeling PGP9.5.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723585, 1/10,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

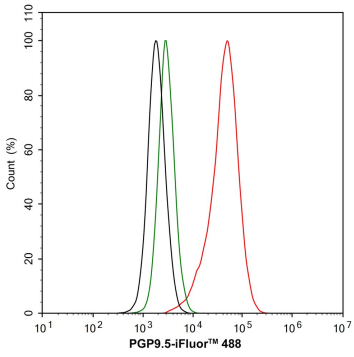

Fig11: Flow cytometric analysis of C6 cells labeling PGP9.5.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723585, 1/10,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Junga A et al. Evaluation of PGP 9.5, NGFR, TGFbeta1, FGFR1, MMP-2, AT2R2, SHH, and TUNEL in Primary Obstructive Megaureter Tissue. J Histochem Cytochem. 2022 Feb
2. Esposito JA et al. A study of PGP 9.5 immunohistochemical labeling on formalin-fixed paraffin embedded tissues for epidermal nerve fiber density testing. J Histotechnol. 2024 Sep