Anti-PGP9.5 Antibody [JM10-59] - BSA and Azide free

HA750375

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC, IHC-Fr
Molecular Wt:	Predicted band size: 25 kDa
Clone number:	JM10-59

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:	Synthetic peptide within Human PGP95 aa 191-223 / 223.
Positive control:	A-172 cell lysate, SHG-44 cell lysate, U-87 MG cell lysate, SH-SY5Y cell lysate, NCI-H1299 cell lysate, A549 cell lysate, 293T cell lysate, Neuro-2a cell lysate, C6 cell lysate, PC-12 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, SH-SY5Y, N2A, PC-12, mouse spinal cord tissue, mouse skin tissue, mouse brain tissue, mouse colon tissue, mouse cerebrum tissue.
Subcellular location:	Cytoplasm, Endoplasmic reticulum membrane.
Database links:	SwissProt: P09936 Human | Q9R0P9 Mouse | Q00981 Rat
Recommended Dilutions:	
WB	1:2,000-1:5,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:1,000
IHC-P	1:1,000-1:5,000
IP	Use at an assay dependent concentration.
FC	1:1,000
IHC-Fr	1:200-1:500
Storage Buffer:	PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

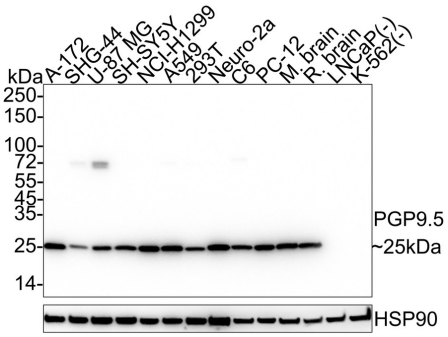

Fig1: Western blot analysis of PGP9.5 on different lysates with Rabbit anti-PGP9.5 antibody (HA750375) at 1/2,000 dilution.

Lane 1: A-172 cell lysate (20 µg/Lane)
Lane 2: SHG-44 cell lysate (20 µg/Lane)
Lane 3: U-87 MG cell lysate (20 µg/Lane)
Lane 4: SH-SY5Y cell lysate (20 µg/Lane)
Lane 5: NCI-H1299 cell lysate (20 µg/Lane)
Lane 6: A549 cell lysate (20 µg/Lane)
Lane 7: 293T cell lysate (20 µg/Lane)
Lane 8: Neuro-2a cell lysate (20 µg/Lane)
Lane 9: C6 cell lysate (20 µg/Lane)
Lane 10: PC-12 cell lysate (20 µg/Lane)
Lane 11: Mouse brain tissue lysate (30 µg/Lane)
Lane 12: Rat brain tissue lysate (30 µg/Lane)
Lane 13: LNCaP cell lysate (negative) (20 µg/Lane)
Lane 14: K-562 cell lysate (negative) (20 µg/Lane)

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

Exposure time: 7 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 (HA750375) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

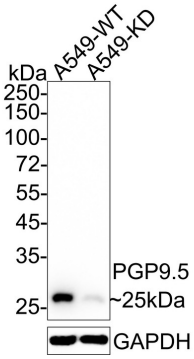

Fig2: Western blot analysis of PGP9.5 on different lysates with Rabbit anti-PGP9.5 antibody (HA750375) at 1/5,000 dilution.

Lane 1: A549-si NT cell lysate (10 µg/Lane)
Lane 2: A549-si PGP9.5 cell lysate (10 µg/Lane)

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

Exposure time: 3 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 (HA750375) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

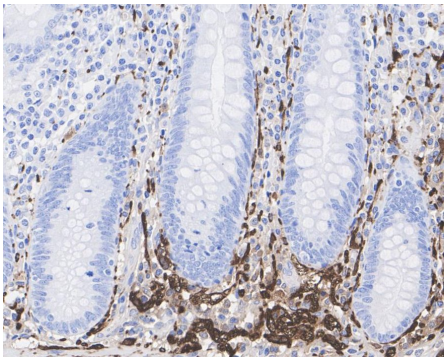

Fig3: Immunohistochemical analysis of paraffin-embedded human appendix tissue with Rabbit anti-PGP9.5 antibody (HA750375) at 1/5,000 dilution.

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 (HA750375) at 1/5,000 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.

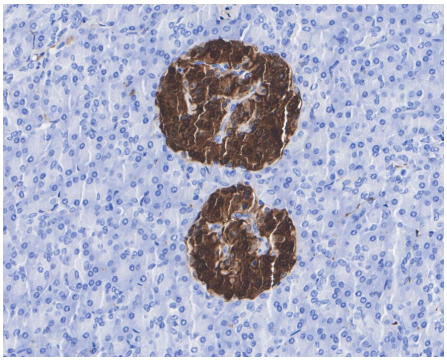

Fig4: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-PGP9.5 antibody (HA750375) at 1/5,000 dilution.

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 (HA750375) at 1/5,000 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.

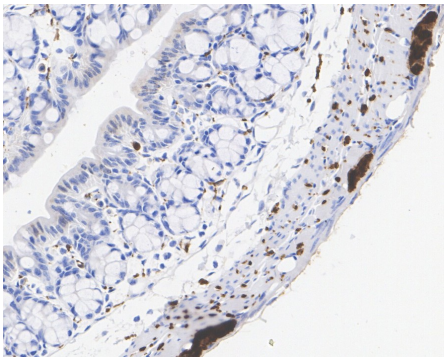

Fig5: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-PGP9.5 antibody (HA750375) at 1/5,000 dilution.

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 (HA750375) at 1/5,000 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.

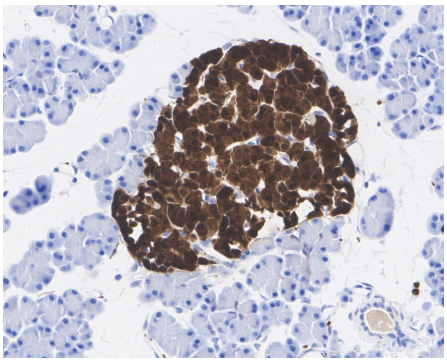

Fig6: Immunohistochemical analysis of paraffin-embedded rat pancreas tissue with Rabbit anti-PGP9.5 antibody (HA750375) at 1/5,000 dilution.

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 (HA750375) at 1/5,000 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.

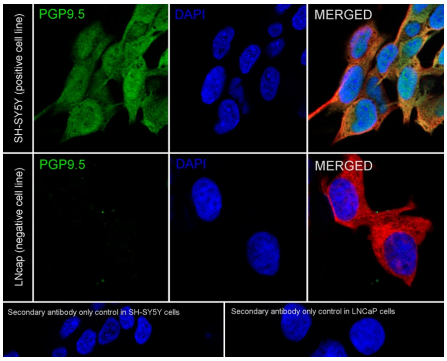

Fig7: Immunocytochemistry analysis of SH-SY5Y (positive) and LNCaP (negative) labeling PGP9.5 with Rabbit anti-PGP9.5 antibody (HA750375) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 (HA750375) 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 (M1305-2, 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.

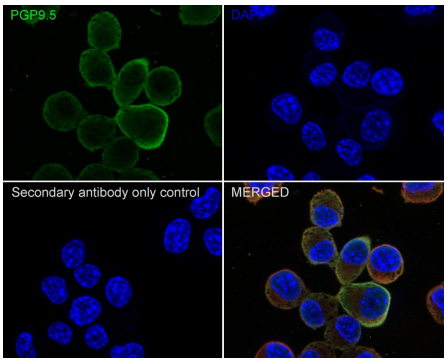

Fig8: Immunocytochemistry analysis of Neuro-2a cells labeling PGP9.5 with Rabbit anti-PGP9.5 antibody (HA750375) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 (HA750375) at 1/100 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 (M1305-2, 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.

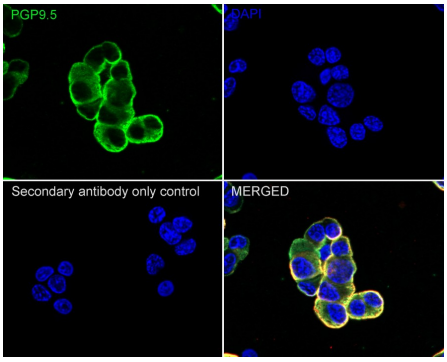

Fig9: Immunocytochemistry analysis of PC-12 cells labeling PGP9.5 with Rabbit anti-PGP9.5 antibody (HA750375) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 (HA750375) at 1/100 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 (M1305-2, 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.

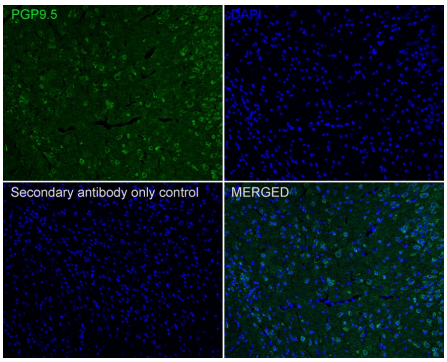

Fig10: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/200

Antigen retrieval: The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven.

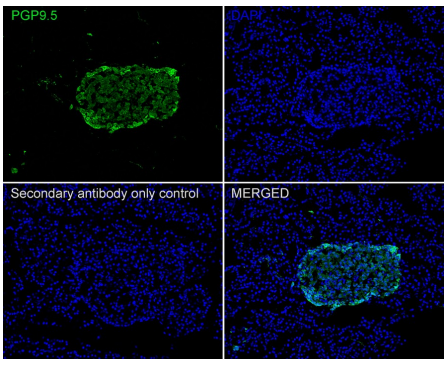

Fig11: Application: IHC-Fr

Species: Mouse

Site: Pancreas

Sample: Frozen section

Antibody concentration: 1/200

Antigen retrieval: The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven.

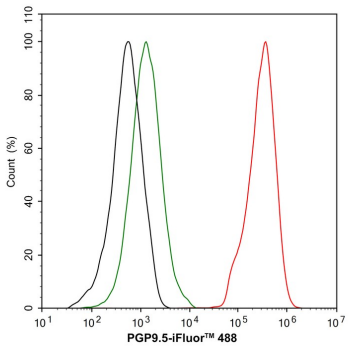

Fig12: Flow cytometric analysis of SH-SY5Y cells labeling PGP9.5.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750375, 1μg/mL) (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).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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