PGP9.5 Recombinant Antibody [JM10-59] - Rat IgG1 (Chimeric)

HA601515

Product Type:	Recombinant Chimeric Antibody, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	IHC-Fr, IF-Tissue, IHC-P, WB, IF-Cell
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: Human pancreas tissue, rat pancreas tissue, SH-SY5Y cell lysate, A-172 cell lysate, Neuro-2a cell lysate, C6 cell lysate, SH-SY5Y.

Subcellular location: Cytoplasm, Endoplasmic reticulum membrane.

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

Recommended Dilutions:

IHC-Fr	1:200
IF-Tissue	1:500
IHC-P	1:2,000
WB	1:10,000
IF-Cell	1:200

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

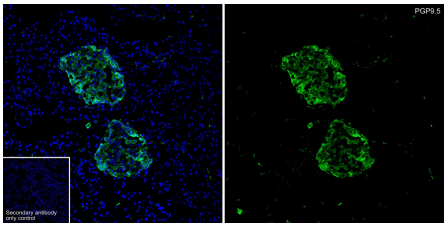


Fig1: Application: IHC-Fr

Species: Rat

Site: pancreas

Sample: Frozen section

Antibody concentration: 1/200

Antigen retrieval: Not required

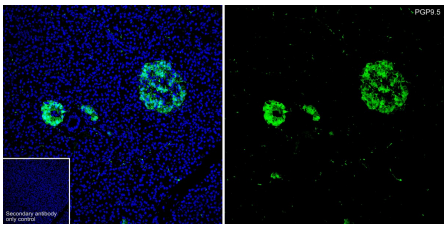


Fig2: Application: IF-Tissue

Species: Human

Site: pancreas

Sample: Paraffin-embedded section

Antibody concentration: 1/500

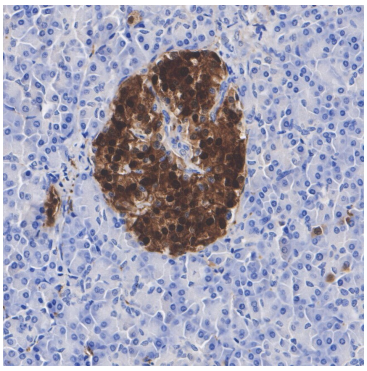


Fig3: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rat anti-PGP9.5 antibody (HA601515) at 1/2,000 dilution.

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 (HA601515) 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.

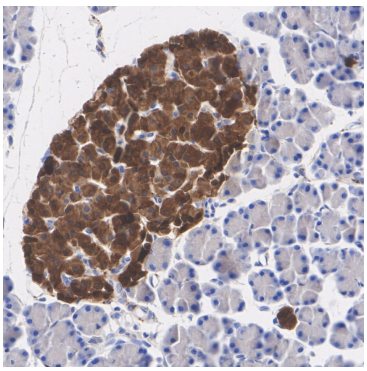


Fig4: Immunohistochemical analysis of paraffin-embedded rat pancreas tissue with Rat anti-PGP9.5 antibody (HA601515) at 1/2,000 dilution.

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 (HA601515) 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.

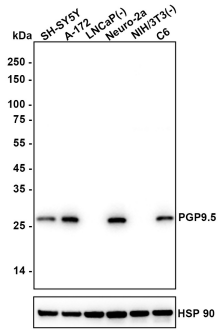


Fig5: Western blot analysis of PGP9.5 on different lysates with Rat anti-PGP9.5 antibody (HA601515) at 1/10,000 dilution.

Lane 1: SH-SY5Y cell lysate
Lane 2: A-172 cell lysate
Lane 3: LNCaP cell lysate (negative)
Lane 4: Neuro-2a cell lysate
Lane 5: NIH/3T3 cell lysate (negative)
Lane 6: C6 cell lysate

Lysates/proteins at 10 µg/Lane.

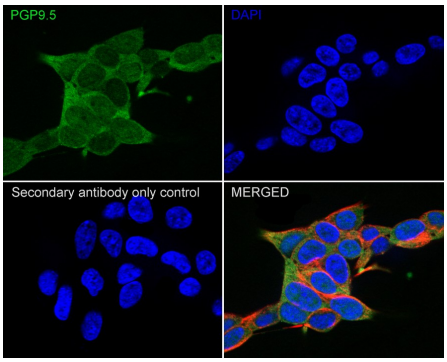
Predicted band size: 25 kDa
Observed band size: 25 kDa

Exposure time: 10 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 (HA601515) at 1/10,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rat IgG H&L - HRP Secondary Antibody (HA1023) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig6: Immunocytochemistry analysis of SH-SY5Y cells labeling PGP9.5 with Rat anti-PGP9.5 antibody (HA601515) at 1/200 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 Rat anti-PGP9.5 antibody (HA601515) at 1/200 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rat IgG H&L (iFluor™ 488, HA1133) was used as the secondary antibody at 1/500 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

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