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Anti-PGP9.5 Antibody Picoband™ (monoclonal, 3E4)

Catalog # ABO15058

Product Information

Application	WB, IHC, FC
Primary Accession	P09936
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-PGP9.5 Antibody Picoband™ (monoclonal, 3E4) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	7345
Other Names	Ubiquitin carboxyl-terminal hydrolase isozyme L1, UCH-L1, 3.4.19.12, Neuron cytoplasmic protein 9.5, PGP 9.5, PGP9.5, Ubiquitin thioesterase L1, UCHL1
Calculated MW	24824
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human, Rat Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 3E4
Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human PGP9.5, different from the related mouse and rat sequences by two amino acids.
Purification	Immunogen affinity purified.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

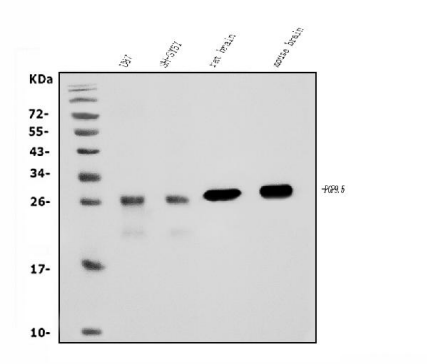
Name	UCHL1
Function	Deubiquitinase that plays a role in the regulation of several processes such as maintenance of synaptic function, cardiac function, inflammatory response or osteoclastogenesis (PubMed:22212137, PubMed:23359680). Abrogates the ubiquitination of multiple proteins including WWTR1/TAZ, EGFR, HIF1A and beta-site amyloid precursor protein cleaving enzyme 1/BACE1 (PubMed:22212137, PubMed:25615526). In addition, recognizes and hydrolyzes a peptide bond at the C-terminal glycine of ubiquitin to maintain a stable pool of monoubiquitin that is a key requirement for the ubiquitin-proteasome and the autophagy- lysosome pathways (PubMed:12408865, PubMed:8639624, PubMed:9774100). Regulates amyloid precursor protein/APP processing by promoting BACE1 degradation resulting in decreased amyloid beta production (PubMed:22212137). Plays a role in the immune response by regulating the ability of MHC I molecules to reach cross-presentation compartments competent for generating Ag-MHC I complexes (By similarity). Mediates the 'Lys-48'-linked deubiquitination of the transcriptional coactivator WWTR1/TAZ leading to its stabilization and inhibition of osteoclastogenesis (By similarity). Deubiquitinates and stabilizes epidermal growth factor receptor EGFR to prevent its degradation and to activate its downstream mediators (By similarity). Modulates oxidative activity in skeletal muscle by regulating key mitochondrial oxidative proteins (By similarity). Enhances the activity of hypoxia-inducible factor 1-alpha/HIF1A by abrogateing its VHL E3 ligase-mediated ubiquitination and consequently inhibiting its degradation (PubMed:25615526).
Cellular Location	Cytoplasm. Endoplasmic reticulum membrane; Lipid- anchor. Note=About 30% of total UCHL1 is associated with membranes in brain. Localizes near and/or within mitochondria to potentially interact with mitochondrial proteins {ECO:0000250|UniProtKB:Q9R0P9}
Tissue Location	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.

Background

UCH-L1, also known as PGP9.5, 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.

Images

Figure 1. Western blot analysis of PGP9.5 using anti-PGP9.5 antibody (M01018-6).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 30ug of sample under reducing conditions.
Lane 1: human U87 whole cell lysates,
Lane 2: human SH-SY5Y whole cell lysates,
Lane 3: rat brain tissue lysates,
Lane 4: mouse brain tissue lysates.



After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-PGP9.5 antigen affinity purified monoclonal antibody (Catalog # M01018-6) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for PGP9.5 at approximately 27KD. The expected band size for PGP9.5 is at 27KD.

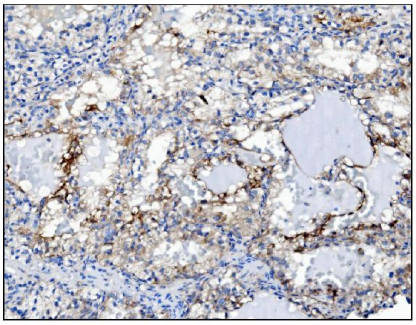


Figure 2. IHC analysis of PGP9.5 using anti-PGP9.5 antibody (M01018-6).
PGP9.5 was detected in paraffin-embedded section of human renal clear cell carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PGP9.5 Antibody (M01018-6) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

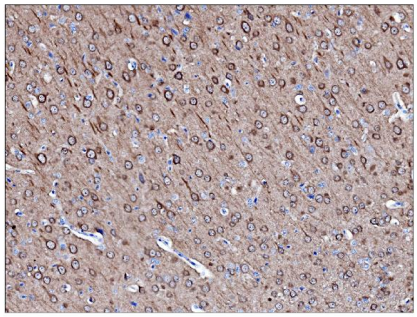


Figure 3. IHC analysis of PGP9.5 using anti-PGP9.5 antibody (M01018-6).
PGP9.5 was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PGP9.5 Antibody (M01018-6) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

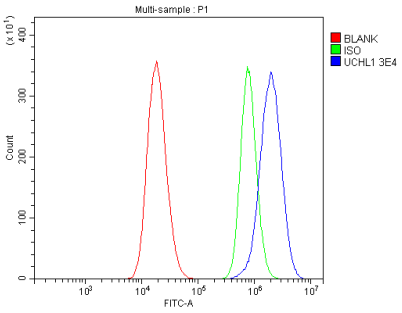


Figure 4. Flow Cytometry analysis of 293T cells using anti-PGP9.5 antibody (M01018-6).
Overlay histogram showing 293T cells stained with M01018-6 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PGP9.5 Antibody (M01018-6, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.