

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

Catalog Number: M01018-6

#### **About UCHL1**

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.

#### Overview

Product Name	Anti-PGP9.5 Antibody Picoband™ (monoclonal, 3E4)
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-PGP9.5 Antibody Picoband™ (monoclonal, 3E4) catalog # M01018-6. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IHC, WB
Clonality	Monoclonal 3E4
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Storage Instructions	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.
Host	Mouse
Uniprot ID	P09936

#### **Technical Details**

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.
Predicted Reactive Species	Hepatitis Virus
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG1
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.



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Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.  If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.  Some PubMed article(s) citing the expression level of this target are as follows:  Boster Bio's internal QC testing used:  Western blot, 0.25-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, Rat Flow Cytometry, 1-3ug/1x10 <sup>6</sup> cells, Human



#### Anti-PGP9.5 Antibody Picoband™ (monoclonal, 3E4) (M01018-6) Images

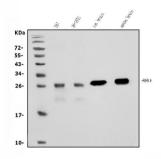


Figure 1. Western blot analysis of PGP9.5 using anti-PGP9.5 antibody (M01018-6).

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 ug/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.

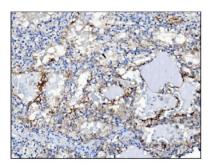


Figure 2. IHC analysis of PGP9.5 using anti-PGP9.5 antibody (M01018-6).

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 2ug/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.

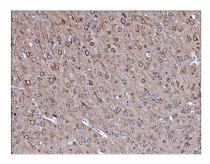


Figure 3. IHC analysis of PGP9.5 using anti-PGP9.5 antibody (M01018-6).

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 2ug/ml mouse anti-PGP9.5 Antibody (M01018-6) overnight at 4°C. Biotinylated goat antimouse 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.

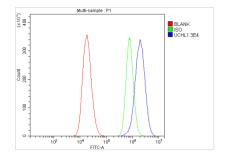


Figure 4. Flow Cytometry analysis of 293T cells using anti-PGP9.5 antibody (M01018-6).

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,  $1ug/1x10^6$  cells) for 30 min at 20°C. DyLight® 488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG ( $1ug/1x10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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