

Anti-ATP5H Antibody Picoband™

Catalog Number: PB9328

About ATP5H

ATP5H is also known as ATPQ. Mitochondrial ATP synthase catalyzes ATP synthesis, utilizing an electrochemical gradient of protons across the inner membrane during oxidative phosphorylation. It is composed of two linked multi-subunit complexes: the soluble catalytic core, F1, and the membrane-spanning component, Fo, which comprises the proton channel. The F1 complex consists of 5 different subunits (alpha, beta, gamma, delta, and epsilon) assembled in a ratio of 3 alpha, 3 beta, and a single representative of the other 3. The Fo seems to have nine subunits (a, b, c, d, e, f, g, F6 and 8). This gene encodes the d subunit of the Fo complex. Alternatively spliced transcript variants encoding different isoforms have been identified for this gene. In addition, three pseudogenes are located on chromosomes 9, 12 and 15.

Overview

Product Name	Anti-ATP5H Antibody Picoband™
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-ATP5H Antibody Picoband™ catalog # PB9328. Tested in IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Application	IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg 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	Rabbit
Uniprot ID	O75947

Technical Details

Immunogen	E.coli-derived human ATP5H recombinant protein (Position: A2-L161). Human ATP5H shares 81% and 78% amino acid (aa) sequence identity with mouse and rat ATP5H, respectively.
Predicted Reactive Species	Bovine
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized

Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
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, 0.1-0.5ug/ml Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, By Heat Immunocytochemistry/Immunofluorescence, 5ug/ml

Anti-ATP5H Antibody Picoband™ (PB9328) Images

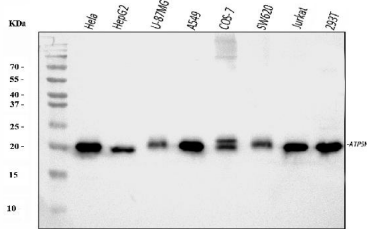


Figure 1. Western blot analysis of ATP5H using anti-ATP5H antibody (PB9328).

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 30 ug of sample under reducing conditions.

Lane 1: human HeLa whole cell lysates,
Lane 2: human HepG2 whole cell lysates,
Lane 3: human U-87MG whole cell lysates,
Lane 4: human A549 whole cell lysates,
Lane 5: monkey COS-7 whole cell lysates,
Lane 6: human SW620 whole cell lysates,
Lane 7: human Jurkat whole cell lysates,
Lane 8: human 293T whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-ATP5H antigen affinity purified polyclonal antibody (Catalog # PB9328) 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-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for ATP5H at approximately 22 kDa. The expected band size for ATP5H is at 22 kDa.

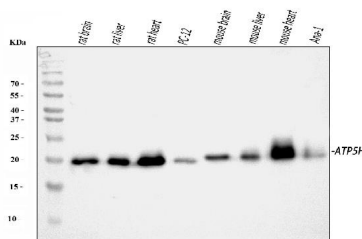


Figure 2. Western blot analysis of ATP5H using anti-ATP5H antibody (PB9328).

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 30 ug of sample under reducing conditions.

Lane 1: rat brain tissue lysates,
Lane 2: rat liver tissue lysates,
Lane 3: rat heart tissue lysates,
Lane 4: rat PC-12 whole cell lysates,
Lane 5: mouse brain tissue lysates,
Lane 6: mouse liver tissue lysates,
Lane 7: mouse heart tissue lysates,
Lane 8: mouse ANA-1 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-ATP5H antigen affinity purified polyclonal antibody (Catalog # PB9328) 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-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for ATP5H at approximately 22 kDa. The

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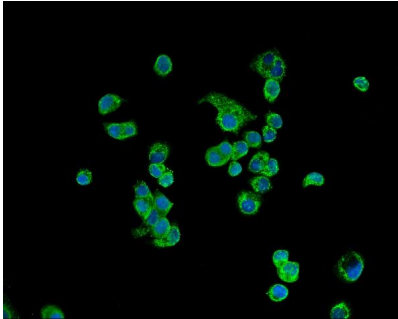


Figure 3. IF analysis of ATP5H using anti-ATP5H antibody (PB9328). ATP5H was detected in an immunocytochemical section of T-47D cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 ug/mL rabbit anti-ATP5H Antibody (PB9328) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

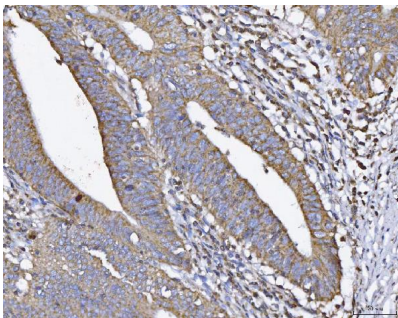


Figure 4. IHC analysis of ATP5H using anti-ATP5H antibody (PB9328). ATP5H was detected in a paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ATP5H Antibody (PB9328) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

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