

Anti-Aquaporin 2/AQP2 Antibody Picoband™

Catalog Number: PB9474

About AQP2

AQP2 (Aquaporin 2), also called AQUAPORIN-CD, is found in the apical cell membranes of the kidney's collecting duct principal cells and in intracellular vesicles located throughout the cell. The AQP2 gene is mapped to chromosome 12q13, very close to the site of major intrinsic protein by situ hybridization. The investigators suggested that a defect in the AQP2 gene is the basis of the autosomal form of nephrogenic diabetes insipidus. The functional expression and the limited localization suggested that AQP2 is the vasopressin-regulated water channel. Using rat kidney slices and porcine kidney cells stably expressing rat Aqp2, AQP2 trafficking can be stimulated by cAMP-independent pathways that utilize nitric oxide (NO). The NO donors sodium nitroprusside (SNP) and NONOate and the NO synthase substrate L-arginine mimicked the effect of vasopressin (VP), stimulating relocation of Aqp2 from cytoplasmic vesicles to the apical plasma membrane. SNP increased intracellular cGMP rather than cAMP, and exogenous cGMP stimulated AQP2 membrane insertion. Atrial natriuretic factor, which signals via cGMP, also stimulated AQP2 translocation. AQP2 expression in kidney connecting tubules is sufficient for survival and that AQP2 expression in collecting ducts is required to regulate body water balance. The S256L substitution in the cytoplasmic tail of the Aqp2 protein prevented phosphorylation at S256 and the subsequent accumulation of Aqp2 on the apical membrane of the collecting duct principal cells.

Overview

Product Name	Anti-Aquaporin 2/AQP2 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Aquaporin 2/AQP2 Antibody Picoband™ catalog # PB9474. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
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	P41181

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human Aquaporin 2, different from the related mouse and rat sequences by one amino acid.
Predicted Reactive Species	Hamster
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).

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, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat, By Heat Flow Cytometry, 1-3ug/1x10⁶ cells, Human

Anti-Aquaporin 2/AQP2 Antibody Picoband™ (PB9474) Images

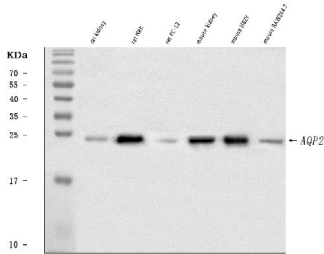


Figure 1. Western blot analysis of Aquaporin 2 using anti-Aquaporin 2 antibody (PB9474).

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 kidney tissue lysates,

Lane 2: rat NRK whole cell lysates,

Lane 3: rat PC-12 whole cell lysates,

Lane 4: mouse kidney tissue lysates,

Lane 5: mouse HBZY whole cell lysates,

Lane 6: mouse RAW264.7 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-Aquaporin 2 antigen affinity purified polyclonal antibody (Catalog # PB9474) 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 Aquaporin 2 at approximately 26 kDa. The expected band size for Aquaporin 2 is at 26 kDa.

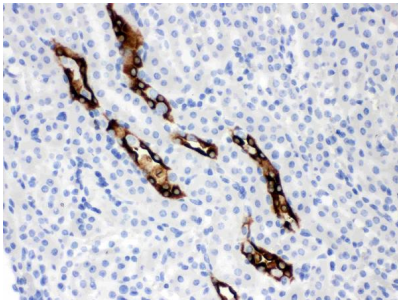


Figure 2. IHC analysis of Aquaporin 2 using anti-Aquaporin 2 antibody (PB9474).

Aquaporin 2 was detected in paraffin-embedded section of Mouse Kidney Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Aquaporin 2 Antibody (PB9474) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

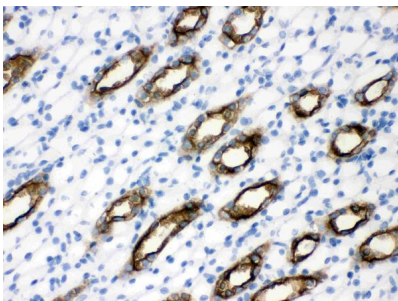


Figure 3. IHC analysis of Aquaporin 2 using anti-Aquaporin 2 antibody (PB9474).

Aquaporin 2 was detected in paraffin-embedded section of Rat Kidney Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Aquaporin 2 Antibody (PB9474) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

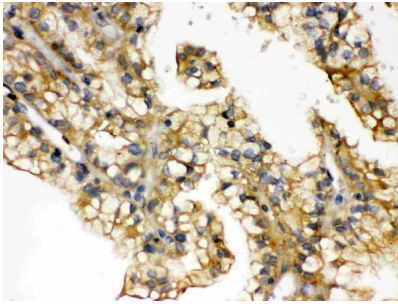


Figure 4. IHC analysis of Aquaporin 2 using anti-Aquaporin 2 antibody (PB9474).

Aquaporin 2 was detected in paraffin-embedded section of Human Renal Cancer Tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Aquaporin 2 Antibody (PB9474) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

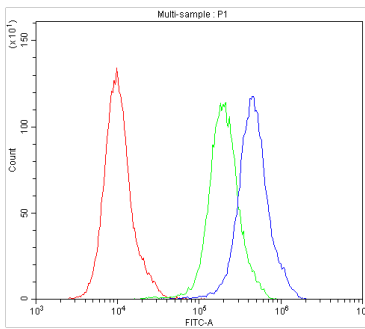


Figure 5. Flow Cytometry analysis of PC-3 cells using anti-AQP2 antibody (PB9474).

Overlay histogram showing PC-3 cells stained with PB9474 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-AQP2 Antibody (PB9474, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

5 Publications Citing This Product

1. PubMed ID: 10.1080/01443615.2017.1327516, Expression and significance of aquaporin-2 and serum hormones in placenta of patients with preeclampsia
2. PubMed ID: 10.1016/j.transproceed.2010.02.070, The Changes of Aquaporin 2 in the Graft of Acute Rejection Rat Renal Transplantation Model
3. PubMed ID: 10.1111/j.1440-1681.2011.05481.x, Effect of exercise training on renal function and renal aquaporin² expression in rats with chronic heart failure

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