Anti-Sodium Potassium ATPase Antibody [ST0533] ET1609-76

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 113 kDa
Clone number:	ST0533

Description: The ubiquitously expressed sodium/potassium-ATPase (Na^+/K^+-ATPase) exists as an oligomeric plasma membrane complex that couples the hydrolysis of one molecule of ATP to the importation of three Na^+ ions and two K^+ ions against their respective electrochemical gradients. As a member of the P-type family of ion motives, Na^+/K^+-ATPase plays a critical role in maintaining cellular volume, resting membrane potential and Na+-coupled solute transport. Multiple isoforms of three subunits, α, β and γ, comprise the Na^+/K^+-ATPase oligomer. The α subunit contains the binding sites for ATP and the cations; the glycosylated β subunit ensures correct folding and membrane insertion of the α subunits. The small γ subunit co-localizes with the α subunit in nephron segments, where it increases the affinity of Na^+/K^+-ATPase for ATP. The β subunit, but not the γ subunit, is essential for normal activity of Na^+/K^+-ATPase.

Immunogen: Synthetic peptide within Human ATP1A1 aa 39-83 / 1023.

Positive control: A549 cell lysates, HeLa cell lysate, HT-29 cell lysate, HepG2 cell lysate, NIH/3T3 cell lysate, L-929 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, HeLa, MCF-7, human liver tissue, human kidney tissue, mouse liver tissue, mouse kidney tissue, rat kidney tissue.

Subcellular location: Cell membrane, Melanosome.

Database links: SwissProt: P05023 Human | P05026 Human | Q13733 Human | P14094 Mouse | Q8VDN2 Mouse | Q9WV27 Mouse | P06685 Rat | P07340 Rat | Q64541 Rat

Recommended Dilutions:

WB	1:50,000-1:100,000
IF-Cell	1:500
IF-Tissue	1:50-1:200
IHC-P	1:50-1:5,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

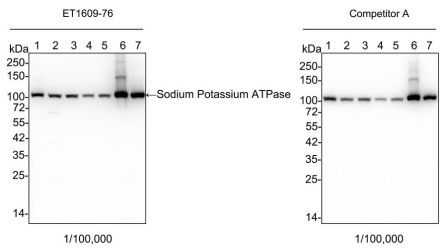

Fig1: Western blot analysis of Sodium Potassium ATPase on different lysates with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/100,000 dilution and competitor's antibody at 1/100,000 dilution.

Lane 1: HeLa cell lysate (no heat) (20 µg/Lane)
Lane 2: HT-29 cell lysate (no heat) (20 µg/Lane)
Lane 3: HepG2 cell lysate (no heat) (20 µg/Lane)
Lane 4: NIH/3T3 cell lysate (no heat) (20 µg/Lane)
Lane 5: L-929 cell lysate (no heat) (20 µg/Lane)
Lane 6: Mouse brain tissue lysate (no heat) (20 µg/Lane)
Lane 7: Rat brain tissue lysate (no heat) (20 µg/Lane)

Predicted band size: 113 kDa
Observed band size: 100 kDa

Exposure time: 20 seconds;

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 (ET1609-76) at 1/100,000 dilution and competitor's antibody at 1/100,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

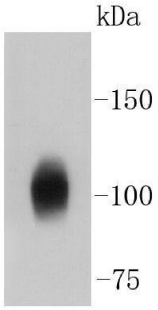

Fig2: Western blot analysis of Sodium Potassium ATPase on A549 cell lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1609-76, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.

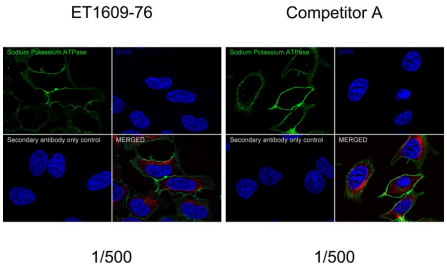

Fig3: Immunocytochemistry analysis of HeLa cells labeling Sodium Potassium ATPase with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/500 dilution and competitor's antibody at 1/500 dilution.

Cells were fixed in 100% precooled methanol for 5 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 Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/500 dilution and competitor's antibody at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

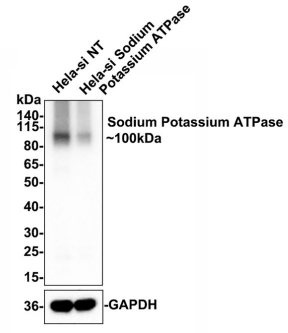

Fig4: Western blot analysis of Sodium Potassium ATPase on different lysates with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/500 dilution.

Lane 1: Hela-si NT cell lysate
Lane 2: Hela-si Sodium Potassium ATPase cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 113 kDa
Observed band size: 100 kDa

Exposure time: 15 seconds;

4-20% SDS-PAGE gel.

ET1609-76 was shown to specifically react with Sodium Potassium ATPase in Hela-si NT cells. Weakened band was observed when Hela-si Sodium Potassium ATPase sample was tested. Hela-si NT and Hela-si Sodium Potassium ATPase samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1609-76, 1/500) and Loading control antibody (Rabbit anti-GAPDH , ET1601-4, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-rabbit IgG-HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

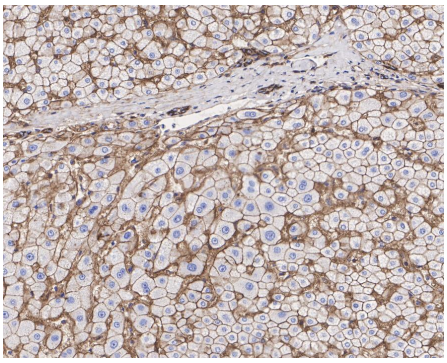

Fig5: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/2,000 dilution.

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 (ET1609-76) 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.

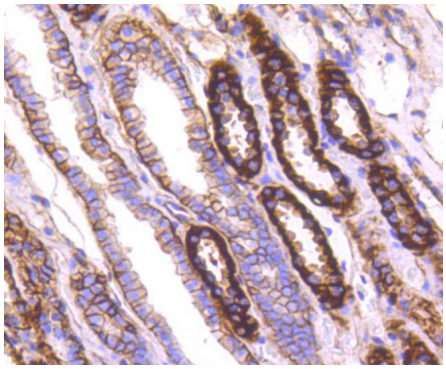

Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-Sodium Potassium ATPase antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-76, 1/50) for 30 minutes 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.

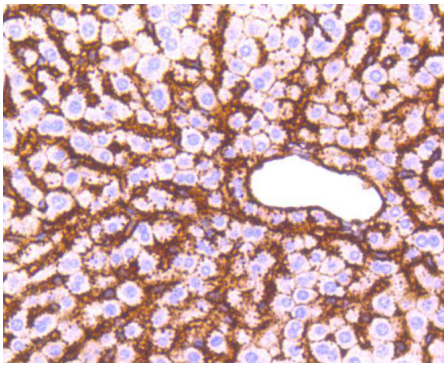

Fig7: Immunohistochemical analysis of paraffin-embedded mouse liver tissue using anti-Sodium Potassium ATPase antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-76, 1/50) for 30 minutes 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.

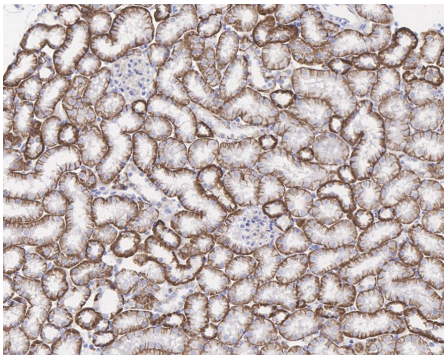

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/5,000 dilution.

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 (ET1609-76) at 1/5,000 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.

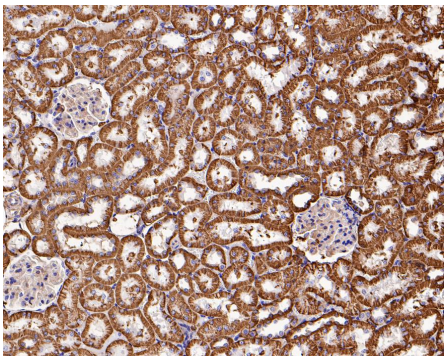

Fig9: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/500 dilution.

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 (ET1609-76) at 1/500 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.

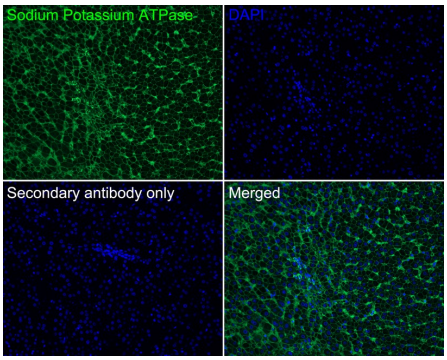

Fig10: Immunofluorescence analysis of paraffin-embedded human liver tissue labeling Sodium Potassium ATPase with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1609-76, green) at 1/200 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

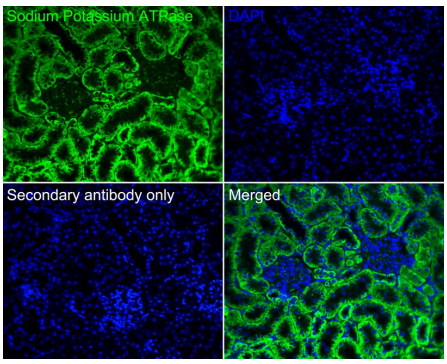

Fig11: Immunofluorescence analysis of paraffin-embedded rat kidney tissue labeling Sodium Potassium ATPase with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1609-76, green) at 1/200 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

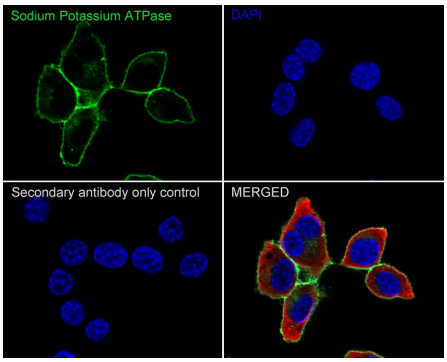

Fig12: Immunocytochemistry analysis of NIH/3T3 cells labeling Sodium Potassium ATPase with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

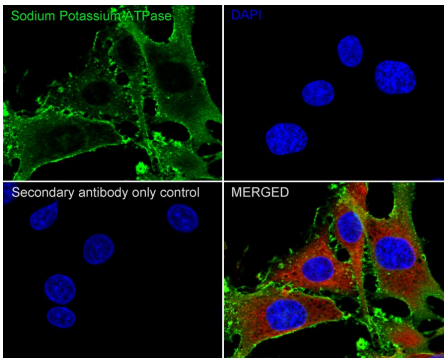

Fig13: Immunocytochemistry analysis of C6 cells labeling Sodium Potassium ATPase with Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Rabbit anti-Sodium Potassium ATPase antibody (ET1609-76) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 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 (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

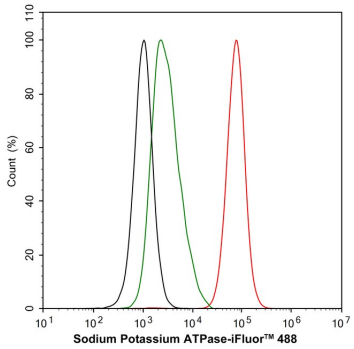

Fig14: Flow cytometric analysis of C6 cells labeling Sodium Potassium ATPase.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1609-76, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Yang SH et al. The lamellae-free-type pseudobranch of the euryhaline milkfish (Chanos chanos) is a Na(+), K(+)-ATPase-abundant organ involved in hypoosmoregulation. Comp Biochem Physiol A Mol Integr Physiol 170:15-25 (2014).
2. R der PV et al. The role of SGLT1 and GLUT2 in intestinal glucose transport and sensing. PLoS One 9:e89977 (2014).