NEUROMICS

ATPase Na+/K+ transporting alpha 1 Data Sheet

Catalog Number:	MO25023	**Host:**	Mouse
Product Type:	Monoclonal, $IgG_{1\ Kappa}$ Clone: 464.6	**Species Reactivity:**	Human, Primate, Mouse, Rat, Cat, Rabbit
Immunogen Sequence:	Purified Na+,K+-ATPase from rabbit renal outer medulla.	**Format:**	50 ul. Ascites Fluid with 0.1% Sodium Azide as a preservative

Applications:
Immunocytochemistry: 1:50-1:200,
Immunofluorescence: 1:100-1:1000,
Immunohistochemistry: 1 :200
Western Blot: 1:1000-1:10000
Flow Cytometry: 1:50-1:200

*Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

Application Notes

This is an excellent plasma membrane marker especially for neurons, glia and related cell types.

Description/Data:
An integral membrane protein complex that hydrolyzes ATP to maintain the transmembrane gradients of Na+ and K+ found in most mammalian cells. The enzyme is comprised of an alpha and beta subunit. The alpha-polypeptide has been shown to be the catalytically active subunit, wherease the Beta-polypeptide appears to be necessary for the assembly and transport of the sodium pump to the plasma membrane. This ATPase Na+/K+ transporting alpha 1 is expressed in both neuronal and non-neuronal cells.

Western Blotting
1. Run ~20 ug of Triton-treated porcine proximal tubule extract protein on a 7.5% SDS-PAGE gel.*
2. Transfer protein to the membrane using a Tris-Glycine/Methanol buffer.
3. Block membrane with TBST/5% NFDM for 30 min. at room temperature (~23-27 degrees C).
4. Wash membrane twice, for 5 minutes each, with TBST.
5. Incubate membrane with 1:5,000 dilution of MO25047 (ATPase Na+/K+ alpha 1), diluted in TBST, for 1 hour at room temperature.
6. Wash membrane once for 15 minutes, then four times for 5 minutes each, with TBST.
7. Incubate membrane with 1:15,000 dilution of goat anti-mouse IgG-HRP [(, diluted in TBST, for 1 hour at room temperature.
8. Wash membrane once for 15 minutes, then four times for 5 minutes each, with TBST.
9. Detect cross-reacting proteins using Chemiluminescent substrate according to manufacturers' guidelines.* NOTE: Do not boil the protein samples, as boiling causes aggregation of the ATPase Na+/K+ alpha 1. The aggregate band will appear at ~150 kDa on Western Blots.

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Immunohistochemistry (FFPE sections)

Deparaffinization:

A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

Quench Endogenous Peroxidase:

A. Place slides in peroxidase quenching solution: 15-30 minutes. To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.

Retrieve Epitopes:

A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

Staining Procedure:

A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
-Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
-All steps in which Xylene is used should be performed in a fume hood.
-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.

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-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 ½ minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker, counterstaining is desired increase time (up to 10 minutes).

Image: Immunohistochemistry: ATPase Na+/K+ alpha 1 staining of enodmetrial glands within the uterus. Note the absence of staining in the surrounding myometrial smooth muscle.

Western Blotting: Detection of ATPase Na+/K+ alpha 1 in in porcine proximal tubule protein.

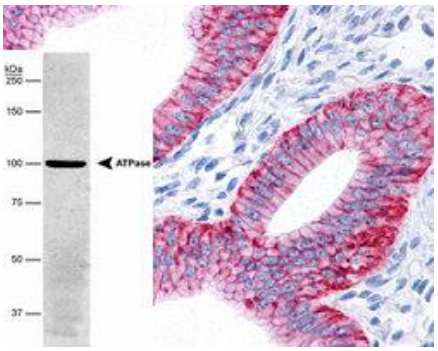
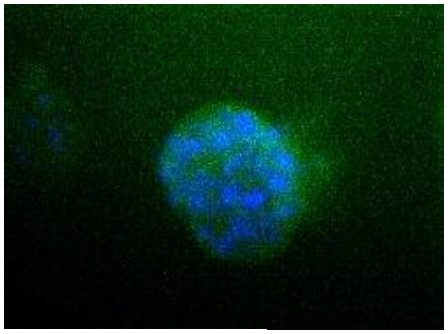
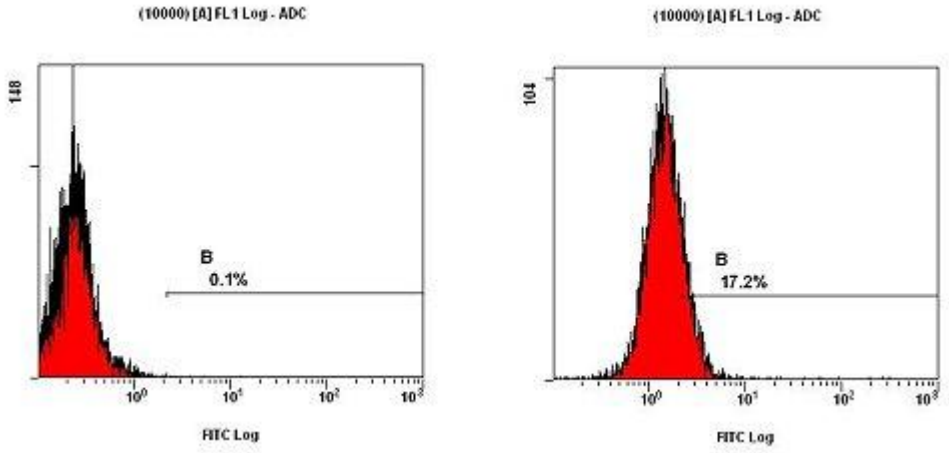


Image: Immunocytochemistry: Detection of ATPA1 (Green) in HepG2 cells using ATPase Na+/K+ alpha 1 .Nuclei (Blue) were counterstained using Hoechst 33258.





Images: Flow Cytometry / FACS analysis: Detection of ATPase Na+/K+ alpha 1 in fixed Hela cells.

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