

# Product Datasheet

## Sodium Potassium ATPase Alpha 1 Antibody (464.6) - BSA Free NB300-146

Unit Size: 0.05 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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**NB300-146**

Sodium Potassium ATPase Alpha 1 Antibody (464.6) - BSA Free

Product Information	
Unit Size	0.05 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	464.6
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	112 kDa

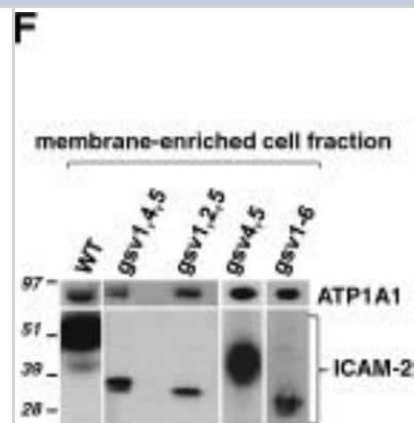
Product Description	
Description	Novus Biologicals Mouse Sodium Potassium ATPase Alpha 1 Antibody (464.6) - BSA Free (NB300-146) is a monoclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-Sodium Potassium ATPase Alpha 1 Antibody: Cited in 115 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	476
Gene Symbol	ATP1A1
Species	Human, Mouse, Rat, Porcine, Bovine, Canine, Drosophila, Guinea Pig, Primate, Rabbit, Sheep, Xenopus, Yeast
Reactivity Notes	Sheep reactivity reported in scientific literature (PMID: 18424241).
Marker	Plasma Membrane Marker
Specificity/Sensitivity	This is specific for Na,K-ATPase alpha 1 subunit.
Immunogen	Purified Sodium Potassium ATPase Alpha 1 from rabbit renal outer medulla. [UniProt# Q9N0Z6]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000-1:10000, Flow Cytometry 1:50-1:200, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:50-1:1000, Immunoprecipitation reported in scientific literature (PMID 27748972), Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen reported in scientific literature (PMID 26941236)
Application Notes	In Western Blot, a distinct band at ~ 112 kDa is seen. Do not boil the sample prior to loading on the gel for Western Blot (60 degrees Celsius appears to work fine).



## Images

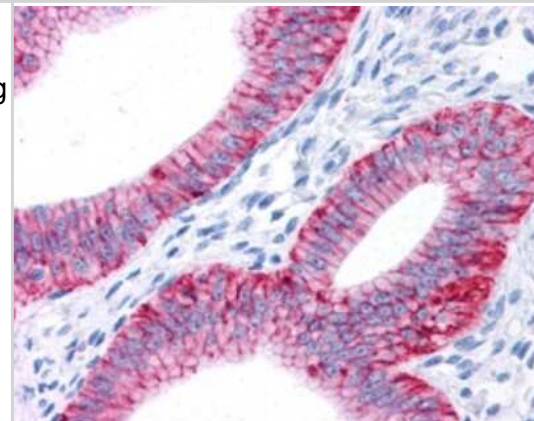
**Western Blot: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146]** - Transfected SK-N-AS cells express ICAM-2 transcripts and proteins. ICAM-2 WT and variants localized to cell membranes. Experimental details are included in Methods. Image collected and cropped by CiteAb from the following publication (<https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-13-261>), licensed under a CC-BY license.



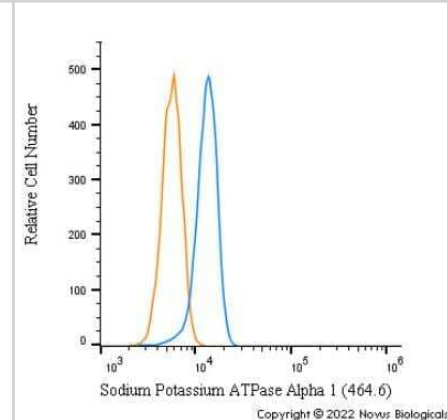
**Immunocytochemistry/Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146]** - The cellular model of the blood-CSF barrier. Immunofluorescent staining of Na<sup>+</sup>K<sup>+</sup> ATPase and ABCC1, showing the expected respective apical and basolateral membrane localization in the choroidal epithelial cells. The left image is a close up of a single cell to better appreciate the polarity of distribution of the 2 proteins. Nuclei appear in blue. Arrows show the lateral cellular membranes best seen in the z direction by confocal analysis, arrowheads show the basal labeling of ABCC1. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0150945>), licensed under a CC-BY license.



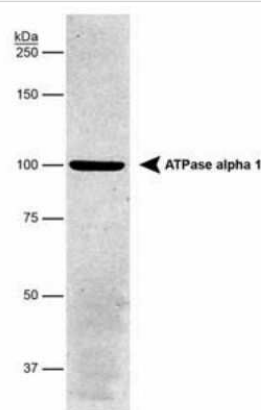
**Immunohistochemistry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146]** - Staining of human endometrial glands within the uterus using NB300-146. Note the absence of staining in the surrounding myometrial smooth muscle.



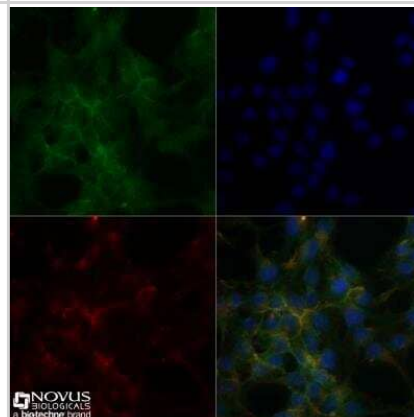
**Flow Cytometry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146]** - An intracellular stain was performed on NIH3T3 cells with Sodium Potassium ATPase Alpha 1 Antibody (464.6) NB300-146 (blue) and a matched isotype control MAB002 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).



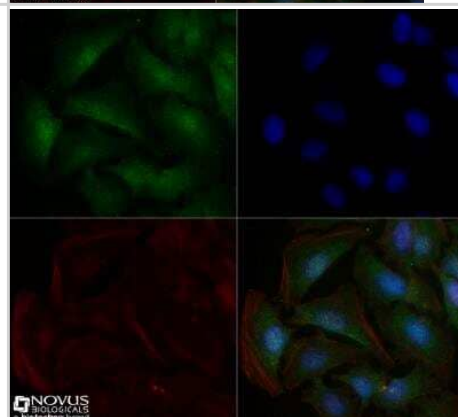
Western Blot: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - Analysis detecting Na, K-ATPase (alpha) in porcine proximal tubule protein.



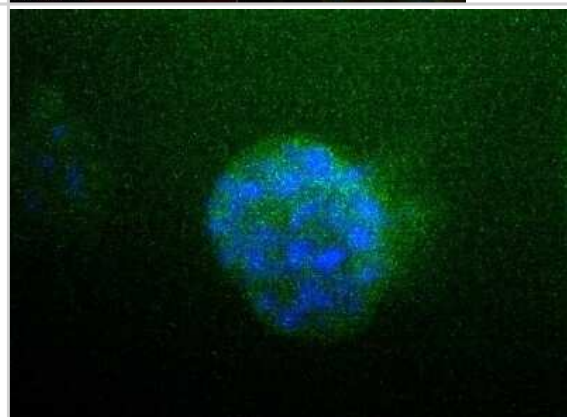
Immunocytochemistry/Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - Hek293 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Sodium Potassium ATPase Alpha 1 (464.6) at 10 ug/ml overnight at 4C and detected with an anti-mouse DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunocytochemistry/Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Sodium Potassium ATPase Alpha 1 (464.6) at 10 ug/ml overnight at 4C and detected with an anti-mouse DyLight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



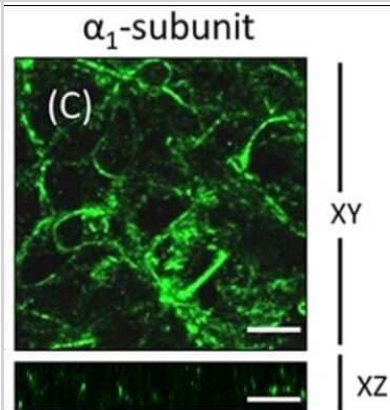
Immunocytochemistry/Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - Detection of ATPA1 (Green) in HepG2 cells using NB300-146. Nuclei (Blue) were counterstained using Hoechst 33258.



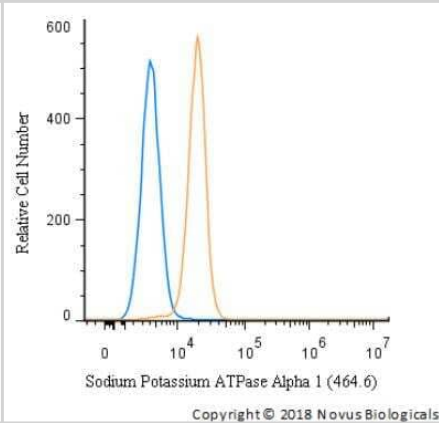
Immunocytochemistry/Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - The cellular model of the blood-CSF barrier. Immunofluorescent staining of Na<sup>+</sup>K<sup>+</sup> ATPase and ABCC1, showing the expected respective apical and basolateral membrane localization in the choroidal epithelial cells. The left image is a close up of a single cell to better appreciate the polarity of distribution of the 2 proteins. Nuclei appear in blue. Arrows show the lateral cellular membranes best seen in the z direction by confocal analysis, arrowheads show the basal labeling of ABCC1. Image collected and cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0150945>), licensed under a CC-BY license.



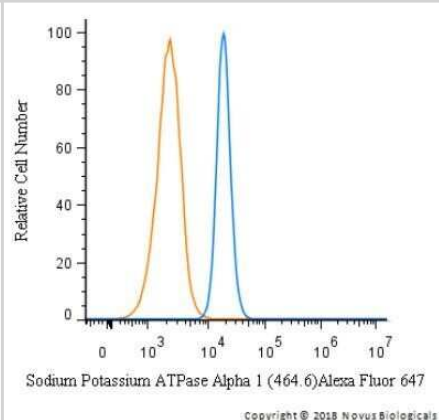
Immunocytochemistry/Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - ARPE-19 cells cultured on transwell inserts for 4 weeks are not completely polarized. ARPE-19 cells were cultured up to 4 weeks on transwell inserts covered with laminin. The expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase using anti-alpha1 antibody was observed mostly at the basolateral membrane. Scale bar: 10  $\mu$ m. Image collected and cropped by CiteAb from the following publication (<https://journal.frontiersin.org/article/10.3389/fphys.2016.00450/full>), licensed under a CC-BY license.



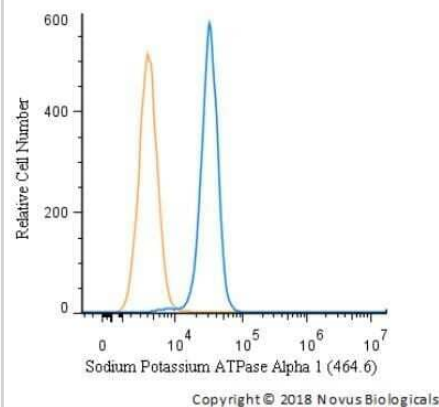
Flow Cytometry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - An intracellular stain was performed on A549 cells with NB300-146 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1  $\mu$ g/mL for 30 minutes at room temperature, followed by mouse F(ab)<sub>2</sub> IgG (H+L) APC-conjugated secondary antibody (F0101B, R&D Systems).



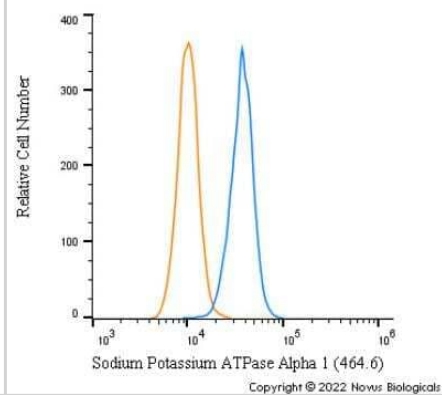
Flow Cytometry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - An intracellular stain was performed on A549 cells with NBP2-61137AF647 (Blue) and a matched isotype control (Orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5  $\mu$ g/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647. Image using the Alexa Fluor 647 form of this antibody.



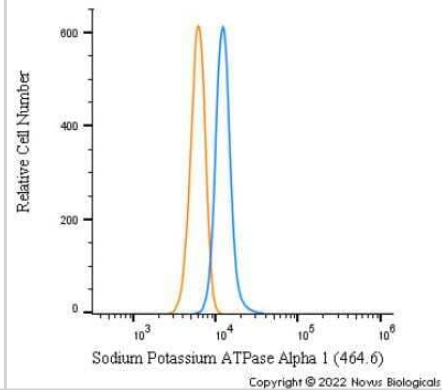
Flow Cytometry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - An intracellular stain was performed on A549 cells with - Purified NBP2-61137 and a matched isotype control. Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) APC-conjugated secondary antibody (F0101B, R&D Systems). Image using the Purified form of this antibody.



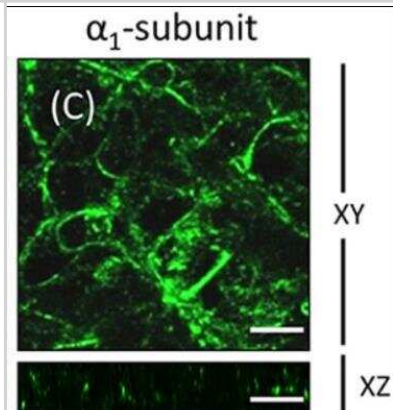
Flow Cytometry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - An intracellular stain was performed on RCC4 cells with Sodium Potassium ATPase Alpha 1 Antibody (464.6) NB300-146 (blue) and a matched isotype control MAB002 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).



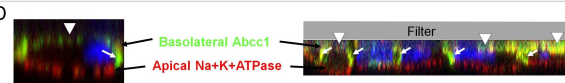
Flow Cytometry: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - An intracellular stain was performed on SK-MEL-28 cells with Sodium Potassium ATPase Alpha 1 Antibody (464.6) NB300-146 (blue) and a matched isotype control MAB002 (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (84540, Thermo Fisher).



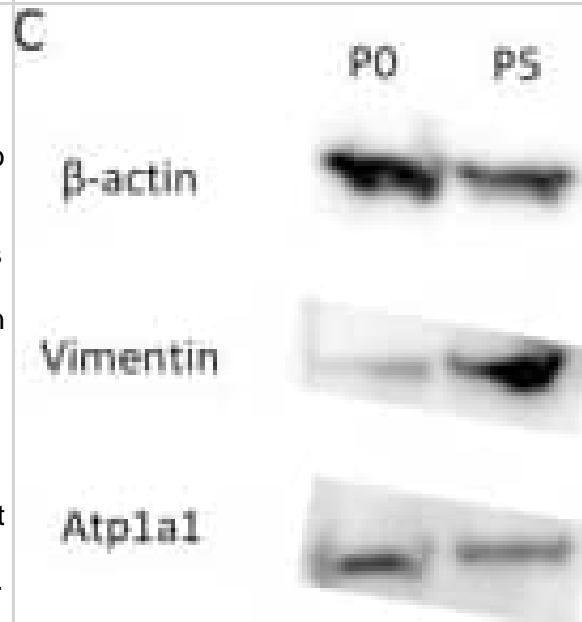
Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - ARPE-19 cells cultured on transwell inserts for 4 weeks are not completely polarized. ARPE-19 cells were cultured up to 4 weeks on transwell inserts covered with laminin. The expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase using anti-alpha1 antibody was observed mostly at the basolateral membrane. Scale bar: 10 um. Image collected and cropped by CiteAb from the following publication (<https://journal.frontiersin.org/article/10.3389/fphys.2016.00450/full>), licensed under a CC-BY license.



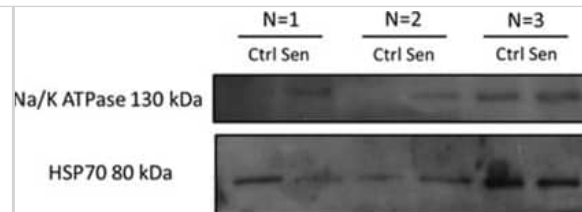
**Immunocytochemistry/ Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146]** - The cellular model of the blood-CSF barrier. (A) Schematic representation of a choroidal villus & of the experimental set up illustrating the two-chamber culture device. Left: the choroidal epithelium which forms the actual tight barrier controlling access into the CSF [18] delimits a stroma, in which the fenestrated vessels lacking a typical blood-brain barrier phenotype, express P-selectin [4]. Right: The epithelial cell monolayer grown on the lower side of the filter separates the upper chamber corresponding to the blood/stromal or basolateral space, from the bottom chamber representing the CSF or apical compartment (adapted from [18,41]). (B & C) Immunofluorescent staining of occludin (B) & claudin 1/3 (C) showing a typical intercellular distribution of the tight junction proteins in the confluent inverted monolayers of choroidal epithelial cells. (D) Immunofluorescent staining of Na+K+ ATPase & ABCC1, showing the expected respective apical & basolateral membrane localization in the choroidal epithelial cells. The left image is a close up of a single cell to better appreciate the polarity of distribution of the 2 proteins. Nuclei appear in blue. Arrows show the lateral cellular membranes best seen in the z direction by confocal analysis, arrowheads show the basal labeling of ABCC1. Image collected & cropped by CiteAb from the following publication (<https://dx.plos.org/10.1371/journal.pone.0150945>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



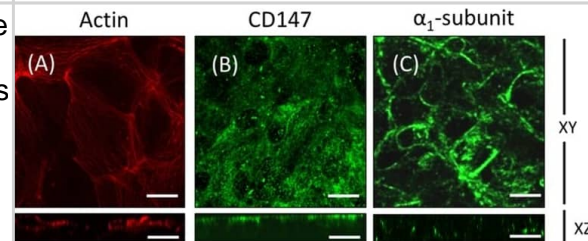
**Western Blot: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146]** - Endothelial-mesenchymal transformation (EMT) of RCECs using specific endothelial-mesenchymal transformation medium (SEMTM). (A) Immunohistochemistry of the fibroblastic markers  $\alpha$ -SMA & vimentin were characteristic of fibroblasts in EMT-RCECs compared to the hexagonal morphology of Fresh RCECs (Scale bar upper panel = 100  $\mu$ m, Scale bar middle & bottom panel = 50  $\mu$ m). (B) RT-PCR showed that both Fresh RCECs & EMT-RCECs expressed  $\alpha$ -Sma. Original gel is shown in Supplemental Fig. S2. However, qRT-PCR in the lower panel shows  $\alpha$ -Sma was significantly upregulated in EMT-RCECs. (C) Western blots showed that Fresh RCECs & EMT-RCECs expressed Vimentin, Atp1a1, a marker of differentiated endothelial cells, & b-actin. Original gel & blots are shown in Supplemental Figs S3, 4. Semiquantitative analysis shows significant upregulation of the protein levels of Vimentin ( $p = 0.048$ ) & downregulation of the protein level of Atp1a1 ( $p = 0.0043$ ). Data expressed as mean  $\pm$  SD of three replicate experiments. Student's t test ( $*p < 0.05$ ,  $**p < 0.01$ ). ( $n = 3$ ). RCECs: rabbit corneal endothelial cells,  $\alpha$ -SMA: smooth muscle  $\alpha$ -actin. Atp1a1: Na,K-ATPase  $\alpha$ -subunit. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/30442918>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - SDS-PAGE western blot validation of the increased abundance of Annexin V, Na/K ATPase &  $\alpha$ -tubulin in the EVs derived from TIS Cal51 cells compared with EVs derived from non-senescent non-treated controls. EVs isolated from TIS & control cells were immunoblotted to validate the protein expression of annexin V, Na/K ATPase &  $\alpha$ -tubulin from the MS data. CD63 & HSP70 were used as loading controls. Densitometry was completed using Image J software (Fiji). All values are expressed as the mean of three independent experiments  $\pm$  s.e.m. The limit of  $\pm 1$  skewness was set as normal distribution. Skewed data were transformed to  $Y = \text{Log}(Y)$  to fit a normal distribution. Results were analysed using the Student's t-test with significant differences having a \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/oncsis201782>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: Sodium Potassium ATPase Alpha 1 Antibody (464.6) [NB300-146] - ARPE-19 cells cultured on transwell inserts for 4 weeks are not completely polarized. ARPE-19 cells were cultured up to 4 weeks on transwell inserts covered with laminin. The immunofluorescence image in (A) shows actin localization using rhodamine phalloidin. The cells are flat with stress fibers & very little circumferential actin microfilament bundles. The expression of CD147, a RPE marker, was detected using a specific antibody in the apical membrane domain (B). The expression of Na<sup>+</sup>, K<sup>+</sup>-ATPase using anti- $\alpha_1$  (C) & anti- $\beta_1$  antibodies (D) was observed mostly at the basolateral membrane. Immunofluorescence detection with anti-human  $\beta_2$  antibody revealed a very weak signal (E). Scale bar: 10  $\mu\text{m}$ . Image collected & cropped by CiteAb from the following publication (<http://journal.frontiersin.org/article/10.3389/fphys.2016.00450/full>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Paparelli L, Corthout N, Pavie B et al. Analyzing Protein Clusters on the Plasma Membrane: Application of Spatial Statistical Analysis Methods on Super-Resolution Microscopy Images Focus on Bio-Image Informatics 2016-05-22 [PMID: 27207364] (Immunohistochemistry, Guinea Pig, Rat)

Yoon S, Myczek K, Penzes P. cAMP Signaling-Mediated Phosphorylation of Diacylglycerol Lipase ? Regulates Interaction With Ankyrin-G and Dendritic Spine Morphology Biological Psychiatry 2021-08-01 [PMID: 34099188] (Immunohistochemistry, Guinea Pig, Rat)

Wang X, Shi J, Li Z et al. An 8-Hydroxy-Quinoline Derivative Protects Against Lipopolysaccharide-Induced Lethality in Endotoxemia by Inhibiting HMGB1-Mediated Caspase-11 Signaling Frontiers in Pharmacology 2021-05-21 [PMID: 34093202] (Immunohistochemistry, Guinea Pig, Rat)

Cheng YS, Zhang T, Ma X et al. A proteome-wide map of 20(S)-hydroxycholesterol interactors in cell membranes Nature Chemical Biology 2021-12-01 [PMID: 34799735] (Immunohistochemistry, Guinea Pig, Rat)

Liu W, Luque M, Li H et al. Spike Generators and Cell Signaling in the Human Auditory Nerve: An Ultrastructural, Super-Resolution, and Gene Hybridization Study Frontiers in Cellular Neuroscience 2021-03-16 [PMID: 33796009] (Immunohistochemistry, Guinea Pig, Rat)

Harich, OO;Gavriliuc, OI;Ordodi, VL;Tirziu, A;Paunescu, V;Panaitescu, C;Bojin, MF; In Vitro Study of the Multimodal Effect of Na<sup>+</sup>/K<sup>+</sup> ATPase Blocker Ouabain on the Tumor Microenvironment and Malignant Cells Biomedicines 2023-08-05 [PMID: 37626702] (Immunohistochemistry, Guinea Pig, Rat)

Wright SS, Kumari P, Fraile-Ágreda V, Wang C et Al. Transplantation of gasdermin pores by extracellular vesicles propagates pyroptosis to bystander cells Cell 2025-01-01 [PMID: 39742811]

Kuintzle R, Santat LA, Elowitz MB. et Al. Diversity in Notch ligand-receptor signaling interactions Elife 2025-01-03 [PMID: 39751380]

Hou P, Zielonka M, Serneels L et al. The  $\gamma$ -secretase substrate proteome and its role in cell signaling regulation Molecular cell 2023-11-16 [PMID: 37977120] (WB, Human)

Details:

1:1000 dilution

Paul D, Stern O, Vallis Y et al. Cell surface protein aggregation triggers endocytosis to maintain plasma membrane proteostasis Nature communications 2023-02-28 [PMID: 36854675] (Immunocytochemistry/ Immunofluorescence, Human)

Rah SY, Joe Y, Park J et al. CD38/ADP-ribose/TRPM2-mediated nuclear Ca<sup>2+</sup> signaling is essential for hepatic gluconeogenesis in fasting and diabetes Experimental & molecular medicine 2023-07-01 [PMID: 37394593] (WB, Mouse)

van der Valk WH, van Beelen ESA, Steinhart MR et al. A single-cell level comparison of human inner ear organoids with the human cochlea and vestibular organs Cell reports 2023-06-06 [PMID: 37289589]

More publications at <http://www.novusbio.com/NB300-146>

## Procedures

### **Immunohistochemistry-Paraffin Protocol for Sodium Potassium ATPase Alpha 1 Antibody (NB300-146)**

#### Immunohistochemistry-Paraffin Embedded Sections

##### Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

##### Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.



**Flow (Intracellular) Protocol for Sodium Potassium ATPase Alpha 1 Antibody (NB300-146)**

## Protocol for Flow Cytometry Intracellular Staining

## Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between  $2 \times 10^5$  and  $1 \times 10^6$  cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100  $\mu$ L for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
  - a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of  $1 \times 10^6$  cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100  $\mu$ L samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

## Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

## Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100  $\mu$ L fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100  $\mu$ L of a permeabilization buffer to every  $1 \times 10^6$  cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
  - a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
  - b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100  $\mu$ L of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1  $\mu$ g per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200  $\mu$ L for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500  $\mu$ L per sample) and proceed with analysis on your flow cytometer.



**Immunocytochemistry/Immunofluorescence Protocol for Sodium Potassium ATPase Alpha 1 Antibody (NB300-146)****Immunocytochemistry Protocol**

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.





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### **Products Related to NB300-146**

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NBL1-07807	Sodium Potassium ATPase Alpha 1 Overexpression Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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