abcam

Product datasheet

Anti-Sodium Potassium ATPase antibody [EP1845Y] -Plasma Membrane Loading Control ab76020

Recombinant RabMAb

★★★★★ 18 Abreviews 235 References 14 Images

Overview

Product name Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control

Description Rabbit monoclonal [EP1845Y] to Sodium Potassium ATPase - Plasma Membrane Loading

Control

Host species Rabbit

Specificity This antibody recognizes an intracellular epitope of Sodium/potassium-transporting ATPase

alpha-1 subunit.

Tested applications Suitable for: ICC/IF, Flow Cyt (Intra), WB, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human, Chinese hamster

Predicted to work with: Tilapia

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, RAW 264.7, CHO, C6, MCF-7, HEK-293 and A431 whole cell lysates; Mouse brain

lysate. IHC-P: Human cervical carcinoma and stomach carcinoma tissues; Mouse liver and lung

tissues; Rat kidney tissue. ICC/IF: T84 cells, MCF-7 cells Flow Cyt (intra): HeLa cells.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EP1845Y

Isotype IgG

Applications

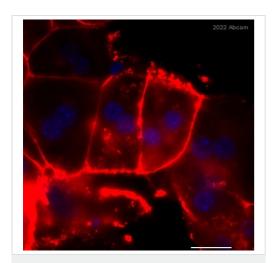
The Abpromise guarantee Our Abpromise guarantee covers the use of ab76020 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (4)	1/500.
Flow Cyt (Intra)		1/20 - 1/100. Follow an intracellular staining protocol. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	★★★★ <u>(5)</u>	1/100000. Predicted molecular weight: 113 kDa. For unpurified, use 1/20000.
IHC-P	★★★★★ (<u>9)</u>	1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.

Target	
Function	This is the catalytic component of the active enzyme, which catalyzes the hydrolysis of ATP coupled with the exchange of sodium and potassium ions across the plasma membrane. This action creates the electrochemical gradient of sodium and potassium ions, providing the energy for active transport of various nutrients.
Sequence similarities	Belongs to the cation transport ATPase (P-type) (TC 3.A.3) family. Type IIC subfamily.
Post-translational modifications	Phosphorylation on Tyr-10 modulates pumping activity.
Cellular localization	Cell membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

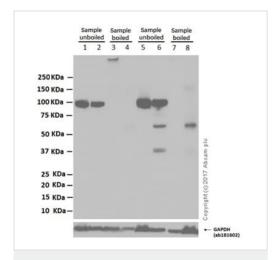
Images



Immunocytochemistry - Anti-Sodium Potassium
ATPase antibody [EP1845Y] - Plasma Membrane
Loading Control (ab76020)

This image is courtesy of an Abreview submitted by Armen Petrosyan

Immunocytochemistry analysis of formaldehyde-fixed rat hepatocytes permeabilized with 0.2% Triton X-100 in PBS, staining with ab76020 at 1/50 dilution. Secondary antibody was Alexa Fluor™ 594 Donkey anti-Rb at 1/200 dilution. Cells were incubated with the primary antibody with 1% donkey serum in PBST for 2 hours at 22°C. Blocking was done with 1% donkey serum in PBST for 1 hour at 22°C.



Western blot - Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020)

All lanes : Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020) at 1/100000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate prepared from RIPA lysis method

Lane 2: HeLa whole cell lysate prepared from 1% SDS HOT lysis method

Lane 3: HeLa whole cell lysate prepared from RIPA lysis method

Lane 4: HeLa whole cell lysate prepared from 1%SDS HOT lysis method

Lane 5: Raw264.7 (Mouse abelson murine leukemia virus-induced tumor) whole cell lysate prepared from RIPA lysis method

Lanes 6 & 8: Raw264.7 whole cell lysate prepared from 1%SDS HOT lysis method

Lane 7 : Raw264.7 whole cell lysate prepared from RIPA lysis method

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

Exposure time: 10 seconds

Blocking/Diluting buffer and concentration 5% NFDM/TBST We suggest not to boil the sample after lysis.

T84 cells (human) cultured on 8-well chamber slides, were washed once with ice-cold PBS, then fixed with 4% paraformaldehyde for 30 min at 4°C. After fixation, cells were permeabilized with 0.5% Triton X-100 for 5 min at room temperature and washed with PBS three times. Following blocking with 2% FCS in PBS for 1 hour at room temperature, primary antibody staining was performed at 4°C overnight at 1/200 dilution. Cells were then incubated with protein fractions B12 and C5 at 5x dilutions in fresh media for 1 hour at 37°C. Cells were then fixed, permeabilized and co-stained with fiber and sodium potassium ATPase. The nuclei were stained with DAPI using Vectachield mounting medium. Cells were visualized using Zeiss confocal microscopy LSM700.

Fiber molecules were found to be predominantly intracellularly following B12 treatment.

For full image see PubMed: 25723153.

Immunocytochemistry/ Immunofluorescence - Anti-Sodium Potassium ATPase antibody [EP1845Y] -

Plasma Membrane Loading Control (ab76020)

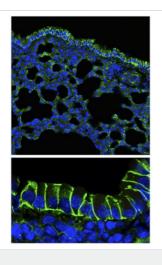
Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Zhang B et al., PLoS One, 10, e0117976, 2015

B12

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020)

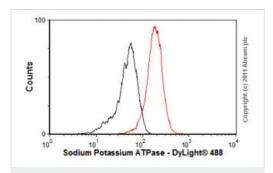
Immunohistochemical staining of paraffin embedded rat kidney with purified ab76020 at a working dilution of 1 in 100. The secondary antibody used is a HRP conjugated goat anti-rabbit IgG (H+L), ab97051, at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was perfored using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020)

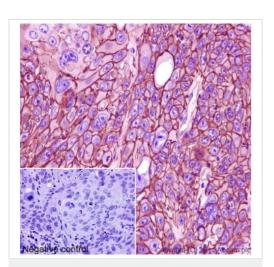
Image from Nieto-Torres JL et al., PLoS Pathog.. 2014;10(5):e1004077. Fig 11.; doi: 10.1371/journal.ppat.1004077 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

ab76020 staining Sodium Potassium ATPase in lung epithelia (top) and bronchiolar epithelia (bottom) from Mouse lung tissue sections by Immunohistochemistry ((IHC) - paraffin-embedded sections). Sections were deparaffined at 60°C and rehydrated by successive incubations in 100% xylol, 100% ethanol and 96% ethanol. Samples were then permeabilized with 0.25% Triton X-100 in PBS for 15 minutes and blocked with 10% bovine serum albumin (BSA) and 0.25% Triton X-100 in PBS for 30 minutes. Samples were incubated with primary antibody (1/100 in 0.25% Triton X-100 and 10% BSA in PBS) for 1 hour 30 minutes at room temperature. An Alexa Fluor®488-conjugated Goat anti-mouse antibody was used as the secondary antibody.



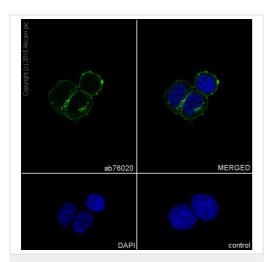
Flow Cytometry (Intracellular) - Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020)

Overlay histogram showing HeLa cells stained with unpurified ab76020 (red line). The cells were fixed with 80% methanol (5 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab76020, 1µg/1x10 6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat antirabbit lgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (1µg/1x10 6 cells) used under the same conditions. Acquisition of >5,000 events was performed.Please note that Abcam do not have any data for use of this antibody on non-fixed cells. We welcome any customer feedback.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium
ATPase antibody [EP1845Y] - Plasma Membrane
Loading Control (ab76020)

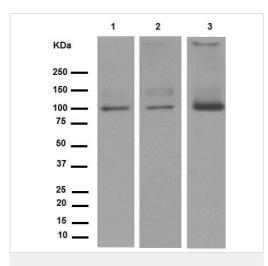
Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified ab76020 at a working dilution of 1 in 100. The secondary antibody used is a HRP conjugated goat anti-rabbit IgG (H+L), ab97051, at a dilution of 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunocytochemistry/ Immunofluorescence - Anti-Sodium Potassium ATPase antibody [EP1845Y] -Plasma Membrane Loading Control (ab76020)

Immunocytochemistry/Immunofluorescence analysis of MCF-7 (human breast carcinoma) cells labelling Sodium Potassium ATPase with purified ab76020 at 1/500. Cells were fixed with 100% methanol. ab150077, Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Nuclei were counterstained with DAPI (blue).

Secondary Only Control: PBS was used instead of the primary antibody as the negative control.



Western blot - Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020)

All lanes : Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020) at 1/100000 dilution

Lane 1: CHO (Chinese hamster ovary cell line) cell lysate

Lane 2: C6 (Rat glial tumor cell line) cell lysate

Lane 3: Mouse brain

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: HRP goat anti-rabbit (H+L) at 1/1000 dilution

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

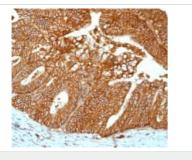
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

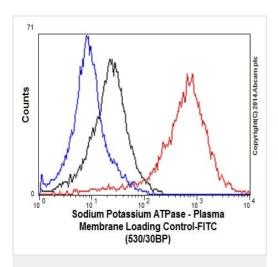
We suggest not to boil the sample after lysis.

Immunohistochemical staining of Sodium Potassium ATPase in paraffin embedded human stomach carcinoma tissue with unpurified ab76020, at a 1/100 dilution.

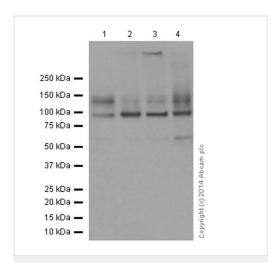
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium
ATPase antibody [EP1845Y] - Plasma Membrane
Loading Control (ab76020)



Flow Cytometry (Intracellular) - Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020) Overlay histogram showingHeLa cells fixed in80% methanoland stained with purified ab76020 at a dilution of 1 in 100 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 150. Rabbit monoclonal IgG was used as an isotype control (black line) and the blue line shows cells incubated without primary or secondary antibody.



Western blot - Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020) **All lanes :** Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020) at 1/100000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2 : MCF-7 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lane 3: HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates

Lane 4: A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/20000 dilution

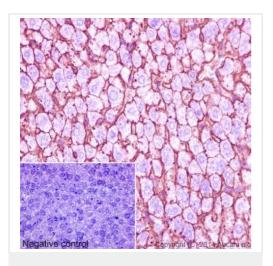
Predicted band size: 113 kDa **Observed band size:** 100 kDa

Exposure time: 2 minutes

Blocking and diluting buffer: 5% NFDM/TBST.

We suggest not to boil the sample after lysis.

Immunohistochemical staining of paraffin embedded mouse liver with purified ab76020 at a working dilution of 1 in 100. The secondary antibody used is a HRP conjugated goat anti-rabbit lgG (H+L), ab97051, at a dilution of 1/500. The sample is counterstained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (ab76020)



(ab76020)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- · Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors