# abcam

# Product datasheet

# Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free ab167390



\*\*\*\* 1 Abreviews 6 References 17 Images

#### Overview

Product name Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free

**Description**Rabbit monoclonal [EP1845Y] to Sodium Potassium ATPase - BSA and Azide free

Host species Rabbit

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

alpha-1 subunit.

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

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

Predicted to work with: Tilapia

Immunogen Synthetic peptide corresponding to Human Sodium Potassium ATPase (N terminal).

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. Flow Cyt (intra): HeLa cells.

**General notes** ab167390 is the carrier-free version of **ab76020**.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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

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- Animal-free production

For more information see here.

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

# **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer Constituent: PBS

Carrier free Yes

Purity Protein A purified

ClonalityMonoclonalClone numberEP1845Y

**Isotype** IgG

# **Applications**

# The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab167390 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
Flow Cyt (Intra)		Use at an assay dependent concentration.  Please refer to the original abID, <u>ab76020</u> , for information on recommended dilutions.
ICC/IF		Use at an assay dependent concentration.  Please refer to the original abID, <u>ab76020</u> , for information on recommended dilutions.
IHC-P	*****(1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.  Please refer to the original abID, <u>ab76020</u> , for information on recommended dilutions.
WB		Use at an assay dependent concentration. Detects a band of approximately 100 kDa (predicted molecular weight: 113 kDa). Please refer to the original abID, <b>ab76020</b> , for information on recommended dilutions.

# **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.

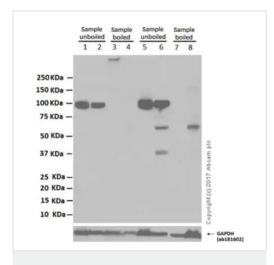
Post-translational modifications

Phosphorylation on Tyr-10 modulates pumping activity.

**Cell ular localization**Cell membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from

stage I to stage IV.

#### **Images**



Western blot - Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free (ab167390)

**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 lgG H&L (HRP) (ab97051)

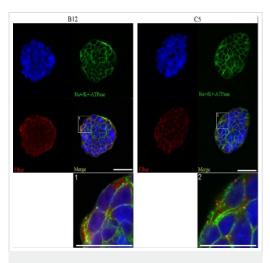
**Predicted band size:** 113 kDa **Observed band size:** 100 kDa

Exposure time: 10 seconds

This data was produced using <u>ab76020</u>, the same antibody clone in a different formulation

Blocking/Diluting buffer and concentration 5% NFDM/TBST

We suggest not to boil the sample after lysis.



Immunocytochemistry/ Immunofluorescence - Anti-Sodium Potassium ATPase antibody [EP1845Y] -BSA and Azide free (ab167390)

Zhang B et al., PLoS One, 10, e0117976, 2015 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/ T84 cells 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.

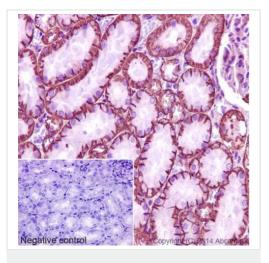
Fiber molecules were found to be predominantly intracellularly following B12 treatment.

For full image see PubMed: 25723153.

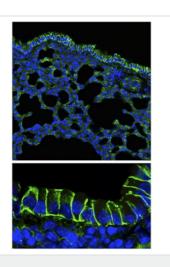
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).

Immunohistochemical staining of paraffin embedded rat kidney with purified <u>ab76020</u> at a working dilution of 1 in 100. The secondary antibody used is a HRP conjugated goat anti-rabbit lgG (H+L), <u>ab97051</u>, 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium
ATPase antibody [EP1845Y] - BSA and Azide free (ab167390)

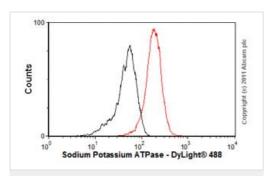


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium
ATPase antibody [EP1845Y] - BSA and Azide free
(ab167390)

Image from Nieto-Torres JL et al. PLoS Pathog. 2014;10(5):e1004077. Fig 11.; doi: 10.1371/journal.ppat.1004077.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).



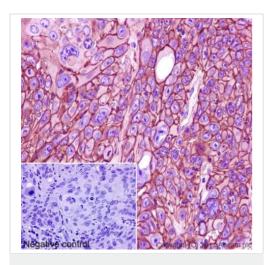
Flow Cytometry (Intracellular) - Anti-Sodium

Potassium ATPase antibody [EP1845Y] - BSA and

Azide free (ab167390)

Overlay histogram showing HeLa cells stained with unpurified  ${\tt ab76020}$  (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 ( ${\tt ab76020}$ , 1µg/1x10 $^6$  cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat antirabbit lgG (H+L) ( ${\tt ab96899}$ ) 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.

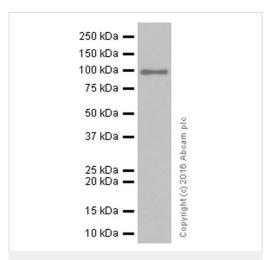
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium
ATPase antibody [EP1845Y] - BSA and Azide free (ab167390)

Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified <u>ab76020</u> at a working dilution of 1 in 100. The secondary antibody used is a HRP conjugated goat anti-rabbit IgG (H+L), <u>ab97051</u>, 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).



Western blot - Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free (ab167390)

Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free (ab167390) + MCF-7 (human breast carcinoma) whole cell lysate at 15  $\mu g$ 

# Secondary

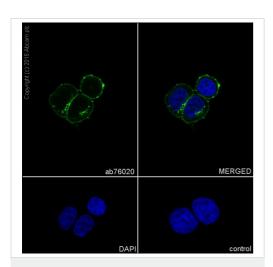
Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

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

Exposure time: 8 seconds

Blocking buffer and concentration: 5% NFDM/TBST

Diluting buffer and concentration: 5% NFDM/TBST

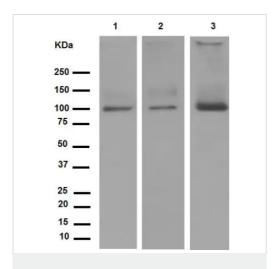


Immunocytochemistry/ Immunofluorescence - Anti-Sodium Potassium ATPase antibody [EP1845Y] -BSA and Azide free (ab167390)

Immunocytochemistry/Immunofluorescence analysis of MCF-7 (human breast carcinoma) cells labelling Sodium Potassium ATPase with purified <u>ab76020</u> at 1/500. Cells were fixed with 100% methanol. <u>ab150077</u>, Alexa Fluor<sup>®</sup> 488-conjugated goat antirabbit lgG (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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).



Western blot - Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free (ab167390)

**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

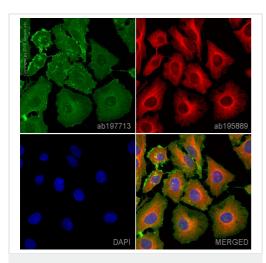
**Predicted band size:** 113 kDa **Observed band size:** 100 kDa

This data was produced using <u>ab76020</u>, the same antibody clone in a different formulation.

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

We suggest not to boil the sample after lysis.



Immunocytochemistry/ Immunofluorescence - Anti-Sodium Potassium ATPase antibody [EP1845Y] -BSA and Azide free (ab167390)

ab198367 ab195887

Immunocytochemistry/ Immunofluorescence - Anti-Sodium Potassium ATPase antibody [EP1845Y] -BSA and Azide free (ab167390)

Clone EP1845Y (ab167390) has been successfully conjugated by Abcam. This image was generated using Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Marker (Alexa Fluor® 488). Please refer to **ab197713** for protocol details.

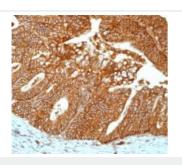
**ab197713** staining Sodium Potassium ATPase in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab197713** at a 1/50 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 594), at a 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Clone EP1845Y (ab167390) has been successfully conjugated by Abcam. This image was generated using Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Marker (Alexa Fluor® 647). Please refer to **ab198367** for protocol details.

<u>ab198367</u> staining Sodium Potassium ATPase in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with <u>ab198367</u> at a 1/100 dilution (shown in red) and <u>ab195887</u>, Mouse monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 488), at a 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

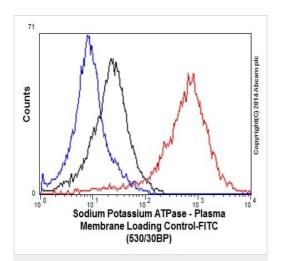
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium
ATPase antibody [EP1845Y] - BSA and Azide free
(ab167390)

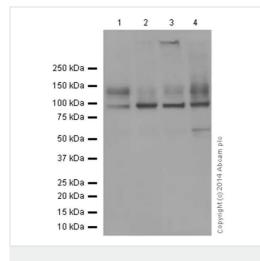
Immunohistochemical staining of Sodium Potassium ATPase in paraffin embedded human stomach carcinoma tissue with unpurified **ab76020**, at a 1/100 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).



Flow Cytometry (Intracellular) - Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free (ab167390) Overlay histogram showing HeLa cells fixed in 80% methanol and stained with purified <a href="mailto:ab76020">ab76020</a> 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 lgG was used as an isotype control (black line) and the blue line shows cells incubated without primary or secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).



Western blot - Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free (ab167390)

**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

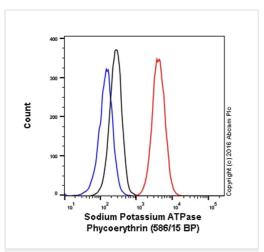
Predicted band size: 113 kDa Observed band size: 100 kDa

Exposure time: 2 minutes

This data was produced using <u>ab76020</u>, the same antibody clone in a different formulation.

Blocking and diluting buffer: 5% NFDM/TBST.

We suggest not to boil the sample after lysis.



Flow Cytometry (Intracellular) - Anti-Sodium

Potassium ATPase antibody [EP1845Y] - BSA and

Azide free (ab167390)

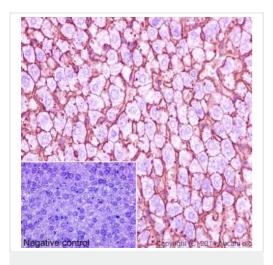
Clone EP1845Y (ab167390) has been successfully conjugated by Abcam. This image was generated using Anti-Sodium Potassium ATPase antibody [EP1845Y] - Plasma Membrane Loading Control (PE). Please refer to <a href="mailto:ab209299">ab209299</a> for protocol details.

Overlay histogram showing HeLa cells stained with <u>ab209299</u> (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (<u>ab209299</u>, 1/2500 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) Phycoerythrin (ab209478) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50mW Yellow-Green laser (561nm) and 586/15 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 4% formaldehyde (10 min)/permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Sodium Potassium
ATPase antibody [EP1845Y] - BSA and Azide free
(ab167390)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76020).



Anti-Sodium Potassium ATPase antibody [EP1845Y] - BSA and Azide free (ab167390)

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

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