abcam

Product datasheet

Anti-Calcium Pump PMCA4 ATPase antibody [JA9] ab2783



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Overview

Product name Anti-Calcium Pump PMCA4 ATPase antibody [JA9]

DescriptionMouse monoclonal [JA9] to Calcium Pump PMCA4 ATPase

Host species Mouse

Specificity This antibody is specific to the PMCA4 isoform.

Tested applications Suitable for: ICC, Flow Cyt, WB, IHC-P

Species reactivity Reacts with: Cow, Human

Immunogen Full length native protein (purified) corresponding to Human Calcium Pump PMCA4 ATPase.

Purified human erythrocyte PMCA.

Positive control WB: SH-SY5Y and HeLa cell lysates.

General notesThe Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 99% PBS

Purity Affinity purified

Clonality Monoclonal

Clone number JA9

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Isotype IgG1

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab2783 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		Use at an assay dependent concentration.	
Flow Cyt		Use 2µg for 10 ⁶ cells. ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.	
WB	★★★★ (1)	1/1000. By Western blot, this antibody detects an ~129 kDa and an ~133 kDa protein representing PMCA4b and PMCA4a ATPase, respectively.	
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.	

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Function This magnesium-dependent enzyme catalyzes the hydrolysis of ATP coupled with the transport of

calcium out of the cell.

Tissue specificity Isoform XB is the most abundant isoform and is expressed ubiquitously. Isoforms containing

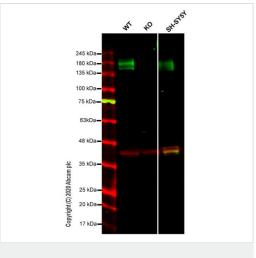
segment Z have only been detected in heart, while isoforms containing segment a have been

found in heart, stomach and brain cortex.

Sequence similaritiesBelongs to the cation transport ATPase (P-type) (TC 3.A.3) family. Type IIB subfamily.

Cellular localization Cell membrane.

Images



Western blot - Anti-Calcium Pump PMCA4 ATPase antibody [JA9] (ab2783)

All lanes : Anti-Calcium Pump PMCA4 ATPase antibody [JA9] (ab2783) at 1/500 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: ATP2B4 knockout HeLa cell lysate

Lane 3: SH-SY5Y cell lysate

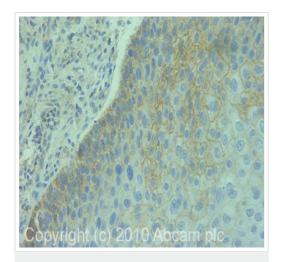
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 140 kDa

Lanes 1-3: Merged signal (red and green). Green - ab2783 observed at 140 kDa. Red - loading control, **ab181602** observed at 37 kDa.

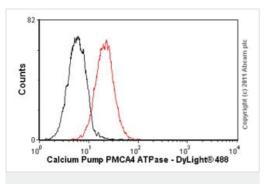
ab2783 Anti-Calcium Pump PMCA4 ATPase antibody [JA9] was shown to specifically react with Calcium Pump PMCA4 ATPase in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab264720 (knockout cell lysate ab258321) was used. Wild-type and Calcium Pump PMCA4 ATPase knockout samples were subjected to SDS-PAGE. ab2783 and Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Calcium Pump PMCA4
ATPase antibody [JA9] (ab2783)

IHC image of ab2783 staining in human cervical carcinoma formalin fixed paraffin embedded tissue section, performed on a Leica BondTM system using the standard protocol F. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab2783, 1µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

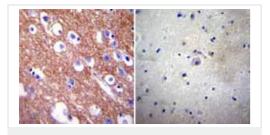
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Flow Cytometry - Anti-Calcium Pump PMCA4 ATPase antibody [JA9] (ab2783)

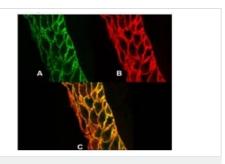
Overlay histogram showing HeLa cells stained with ab2783 (red line). The cells were fixed with 80% methanol (5 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block nonspecific protein-protein interactions. The cells were then incubated with the antibody (ab2783, 2µg/1x10 6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse lgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG1 [ICIGG1] (ab91353, 2µg/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-Calcium Pump PMCA4
ATPase antibody [JA9] (ab2783)

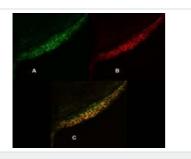
Immunohistochemistry was performed on normal biopsies of deparaffinized Human brain tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing PMCA4 ATPase ab2783 or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Immunocytochemistry - Anti-Calcium Pump PMCA4
ATPase antibody [JA9] (ab2783)

Immunolocalization of Calcium Pump PMCA4 ATPase in human corneal epithelium.

- (A) Immunolocalization of PMCA4 (green) using ab2783 on cell membranes of all cells in each layer of human corneal epithelium.
- (B) Immunolocalization of Calcium Sensing Receptor (red) using <u>ab19347</u> on cell membranes of human corneal epithelium.
- (C) Merged image.



Immunocytochemistry - Anti-Calcium Pump PMCA4
ATPase antibody [JA9] (ab2783)

Immunolocalization of Calcium Pump PMCA4 and Calcium Sensing Receptor in bovine corneal epithelium (bCE).

- (A) Immunolocalization of Calcium Pump PMCA4 (green) using ab2783 on cell membranes of all cells in each layer of bCE.
- (B) Immunolocalization of Calcium Sensing Receptor (red) using <u>ab19347</u> on cell membranes of bCE mostly in wing- and squamous cell layers.
- (C) Merged image.

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