abcam

Product datasheet

Anti-SERCA2 ATPase antibody [2A7-A1] ab2861

27 Abreviews 86 References 10 Images

Overview

Product name	Anti-SERCA2 ATPase antibody [2A7-A1]
Description	Mouse monoclonal [2A7-A1] to SERCA2 ATPase
Host species	Mouse
Tested applications	**Suitable for:** WB, IHC-Fr, ICC/IF, ICC, IP, IHC-P, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Sheep, Rabbit, Guinea pig, Dog, Human, Pig, Xenopus laevis **Predicted to work with:** Chicken, Cat
Immunogen	Full length native protein (purified) corresponding to Dog SERCA2 ATPase. Purified from canine cardiac sarcoplasmic reticulum vesicles.
Epitope	The epitope this antibody recognizes (amino acids 386-396) which is present in both isoforms (SERCA2a and SERCA2b).
Positive control	ICC/IF: U-251 MG, A549 and HeLa cells. IHC-P: Human left ventricle, skeletal muscle, tonsil and liver tissue. Flow Cyt: HepG2 cells.
General notes	**Abcam is committed to meeting high quality standards of ethical manufacturing and has decided to discontinue this product by June 2020 as it has been generated by the ascites method. We are sorry for any inconvenience this may cause. We suggest ab150435 or ab137020 or ab3625 as possible replacements.**

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 Constituent: PBS
Purity	Ascites
Primary antibody notes	ATP dependent calcium pumps are responsible in part for the maintenance of low cytoplasmic free calcium concentrations. The ATP pumps that reside in intracellular organelles are comprised of a family of structurally related enzymes, termed the sarcoplasmic or endoplasmic reticulum calcium (SERCA) ATPases. The SERCA2 gene is subject to tissue dependent processing which is responsible for the generation of SERCA2a muscle-specific isoform expressed in type I (slow)

skeletal, cardiac and smooth muscle and the SERCA2b isoform expressed in all cell types. The SERCA3 gene is not as well characterized and is found in non-muscle cells.

Clonality	Monoclonal
Clone number	2A7-A1
Isotype	IgG2a

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab2861 in the following tested applications.

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

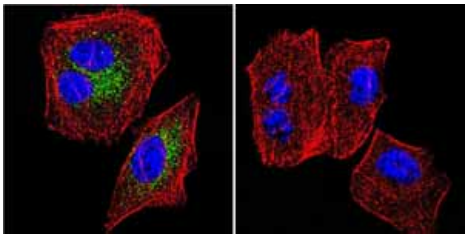
Application	Abreviews	Notes
WB	★★★★★ (19)	1/1000. Detects a band of approximately 110 kDa (predicted molecular weight: 110 kDa). A lower background band at ~80 kDa may also be detected. Blocking conditions will need to be optimized.
IHC-Fr	★★★★★ (1)	1/100.
ICC/IF	★★★★★ (6)	Use at an assay dependent concentration.
ICC	★★★★★ (1)	1/250.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

Target

Function	This magnesium-dependent enzyme catalyzes the hydrolysis of ATP coupled with the translocation of calcium from the cytosol to the sarcoplasmic reticulum lumen. Isoform 2 is involved in the regulation of the contraction/relaxation cycle.
Tissue specificity	Isoform 1 is widely expressed in smooth muscle and nonmuscle tissues such as in adult skin epidermis, with highest expression in liver, pancreas and lung, and intermediate expression in brain, kidney and placenta. Also expressed at lower levels in heart and skeletal muscle. Isoforms 2 and 3 are highly expressed in the heart and slow twitch skeletal muscle. Expression of isoform 3 is predominantly restricted to cardiomyocytes and in close proximity to the sarcolemma. Both isoforms are mildly expressed in lung, kidney, liver, pancreas and placenta. Expression of isoform 3 is amplified during monocytic differentiation and also observed in the fetal heart.
Involvement in disease	Defects in ATP2A2 are a cause of acrokeratosis verruciformis (AKV) [MIM:101900]; also known as Hopf disease. AKV is a localized disorder of keratinization, which is inherited as an autosomal dominant trait. Its onset is early in life with multiple flat-topped, flesh-colored papules on the hands

	and feet, punctate keratoses on the palms and soles, with varying degrees of nail involvement. The histopathology shows a distinctive pattern of epidermal features with hyperkeratosis, hypergranulosis, and acanthosis together with papillomatosis. These changes are frequently associated with circumscribed elevations of the epidermis that are said to resemble church spires. There are no features of dyskeratosis or acantholysis, the typical findings in lesions of Darier disease. Defects in ATP2A2 are the cause of Darier disease (DD) [MIM:124200]; also known as Darier-White disease (DAR). DD is an autosomal dominantly inherited skin disorder characterized by loss of adhesion between epidermal cells (acantholysis) and abnormal keratinization. Patients with mild disease may have no more than a few scattered keratotic papules or subtle nail changes, whereas those with severe disease are handicapped by widespread malodorous keratotic plaques. In a few families, neuropsychiatric abnormalities such as mild mental retardation, schizophrenia, bipolar disorder and epilepsy have been reported. Stress, UV exposure, heat, sweat, friction, and oral contraception exacerbate disease symptoms. Prevalence has been estimated at 1 in 50000. Clinical variants of DD include hypertrophic, vesicobullous, hypopigmented, cornifying, zosteriform or linear, acute and comedonal subtypes. Comedonal Darier disease (CDD) is characterized by the coexistence of acne-like comedonal lesions with typical Darier hyperkeratotic papules on light-exposed areas. At histopathologic level, CDD differs from classic DD in the prominent follicular involvement and the presence of greatly elongated dermal villi.
Sequence similarities	Belongs to the cation transport ATPase (P-type) (TC 3.A.3) family. Type IIA subfamily.
Post-translational modifications	Nitrated under oxidative stress. Nitration on the two tyrosine residues inhibits catalytic activity.
Cellular localization	Endoplasmic reticulum membrane. Sarcoplasmic reticulum membrane.

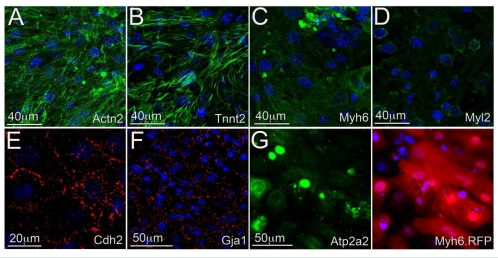
Images



Immunocytochemistry/ Immunofluorescence - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

Immunofluorescent analysis of SERCA2 ATPase using SERCA2 ATPase Monoclonal antibody (2A7-A1) ab2861 shows staining in U-251 MG (Human brain glioma cell line) cells.

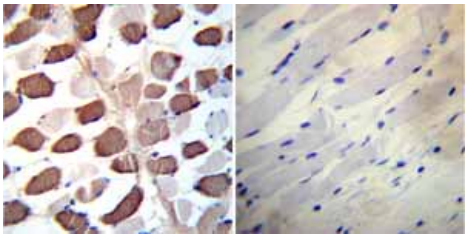
SERCA2 ATPase staining (green) F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing SERCA2 ATPase ab2861 at a dilution of 1:100-1:200 over night at 4°C washed with PBS and incubated with a DyLight®-488 conjugated secondary antibody. Images were taken at 60X magnification.



Immunocytochemistry/ Immunofluorescence - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

Immunofluorescence characterization of cardiomyocytes differentiated from the cardiac progenitor cells.

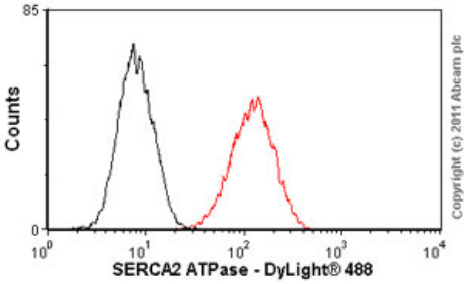
A–D. The cardiomyocytes formed well-defined cross-striated sarcomeric structures as determined by the expression and spatial organization of Actn2, Tnnt2, and Myh6 and also expressed the ventricular specific protein Myl2. **E–F**. The cells also formed robust intercellular electrical and mechanical connections as determined by the spatial organization of Cdh2 and Gja1. **G**. RFP(+) cardiomyocytes stained positive for sodium/potassium ATPase (Atp2a2) using ab2861.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized human skeletal muscle tissues.

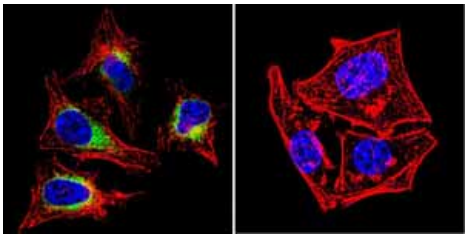
To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH 6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing SERCA2 ATPase ab2861 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.



Flow Cytometry - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

Overlay histogram showing HepG2 (Human liver hepatocellular carcinoma cell line) cells stained with ab2861 (red line).

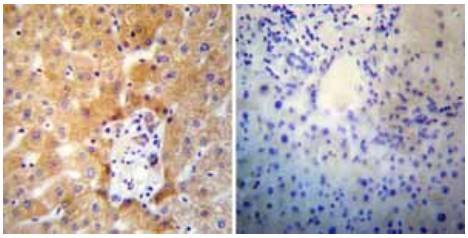
The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab2861, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) ab96879 at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunocytochemistry/ Immunofluorescence - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

Immunofluorescent analysis of SERCA2 ATPase using SERCA2 ATPase Monoclonal antibody (2A7-A1) ab2861 shows staining in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

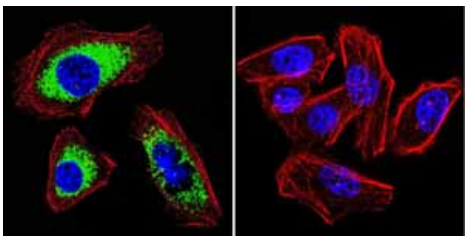
SERCA2 ATPase staining (green) F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing SERCA2 ATPase ab2861 at a dilution of 1:100-1:200 over night at 4°C washed with PBS and incubated with a DyLight®488 conjugated secondary antibody. Images were taken at 60X magnification.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized human liver tissue tissues.

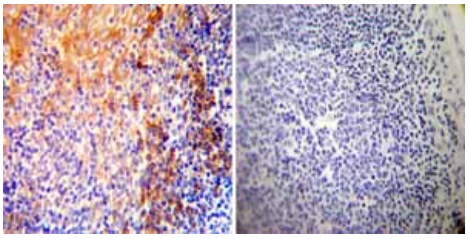
To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH 6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:100 with a mouse monoclonal antibody recognizing SERCA2 ATPase ab2861 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/ Immunofluorescence - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

Immunofluorescent analysis of SERCA2 ATPase using SERCA2 ATPase Monoclonal antibody (2A7-A1) ab2861 shows staining in A549 (Human lung carcinoma cell line) cells.

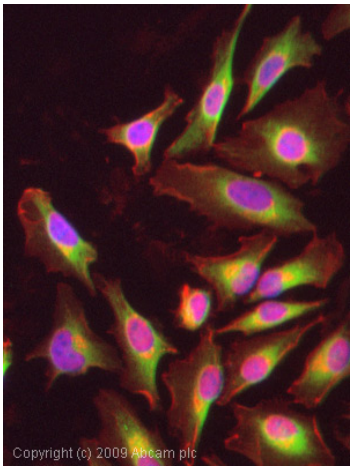
SERCA2 ATPase staining (green) F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with or an antibody recognizing SERCA2 ATPase ab2861 at a dilution of 1:100-1:200 over night at 4°C washed with PBS and incubated with a DyLight®488 conjugated secondary antibody. Images were taken at 60X magnification.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

Immunohistochemistry was performed on both normal and cancer biopsies of deparaffinized human tonsil tissue tissues.

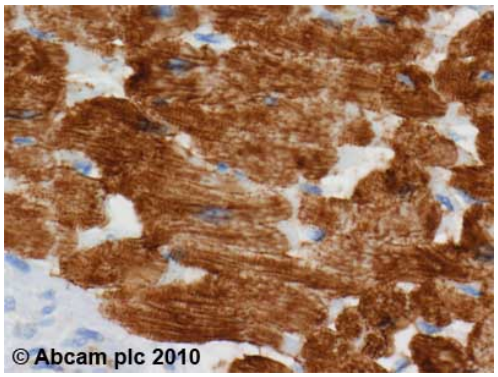
To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH 6.0) buffer microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a mouse monoclonal antibody recognizing SERCA2 ATPase ab2861 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/ Immunofluorescence - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

ICC/IF image of ab2861 stained HeLa (Human epithelial cell line from cervix adenocarcinoma cells.

The cells were fixed with 4% PFA (10 min) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab2861, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SERCA2 ATPase antibody [2A7-A1] (ab2861)

ab2861 (1 µg/ml) staining SERCA2 ATPase in human left ventricle using an automated system (DAKO Autostainer Plus). Using this protocol there is strong cytoplasmic staining of cardiomyocytes. Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer EDTA pH 9.0 in a DAKO PT link. Slides were blocked in 3% H_2O_2/methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with hematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.

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