abcam

Product datasheet

Anti-SDHA antibody [2E3GC12FB2AE2] ab14715



★★★★★ 25 Abreviews 416 References 8 Images

Overview

Product name Anti-SDHA antibody [2E3GC12FB2AE2]

Description Mouse monoclonal [2E3GC12FB2AE2] to SDHA

Host species Mouse

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

Species reactivity Reacts with: Mouse, Rat, Cow, Human

Predicted to work with: Dog

Immunogen Full length native protein (purified). This information is considered to be commercially sensitive.

Positive control WB: HEK-293, MCF7 amd HEepG2 whole cell lysates; Mitochondria isolated from human heart,

cow heart, rat heart, mouse heart and HepG2 cells. Flow cyt: HL-60 cells. IHC-P: Human testis and skeletal muscle tissue. ICC: Cultured human embryonic lung-derived fibroblasts (strain

MRC5).

General notes

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or

conjugation for your experiments, please contact orders@abcam.com.

The 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

Product was previously marketed under the MitoSciences sub-brand.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C.

Storage buffer pH: 7.5

Preservative: 0.02% Sodium azide Constituent: HEPES buffered saline

1

Purity IgG fraction

Purification notes Near homogeneity as judged by SDS-PAGE. The antibody was produced in vitro using

hybridomas grown in serum-free medium, and then purified by biochemical fractionation.

Clonality Monoclonal

Clone number 2E3GC12FB2AE2

Light chain type lgG1 kappa

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab14715 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
IHC-Fr		Use at an assay dependent concentration.
Flow Cyt	★★★★ (1)	Use a concentration of 1 μ g/ml. <u>ab170190</u> - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
WB	**** (17)	Use a concentration of 0.1 µg/ml. Detects a band of approximately 70 kDa (predicted molecular weight: 70 kDa).
ICC		Use at an assay dependent concentration.
IHC-P	★★★★★ (<u>5)</u>	Use at an assay dependent concentration.

Target

Function Flavoprotein (FP) subunit of succinate dehydrogenase (SDH) that is involved in complex II of the

mitochondrial electron transport chain and is responsible for transferring electrons from succinate

to ubiquinone (coenzyme Q).

Pathway Carbohydrate metabolism; tricarboxylic acid cycle; fumarate from succinate (eukaryal route): step

1/1.

Involvement in disease Defects in SDHA are a cause of mitochondrial complex II deficiency (MT-C2D) [MIM:252011]. A

disorder of the mitochondrial respiratory chain with heterogeneous clinical manifestations. Clinical features include psychomotor regression in infants, poor growth with lack of speech development, severe spastic quadriplegia, dystonia, progressive leukoencephalopathy, muscle weakness, exercise intolerance, cardiomyopathy. Some patients manifest Leigh syndrome or Kearns-Sayre

syndrome.

Defects in SDHA are a cause of Leigh syndrome (LS) [MIM:256000]. LS is a severe disorder

characterized by bilaterally symmetrical necrotic lesions in subcortical brain regions.

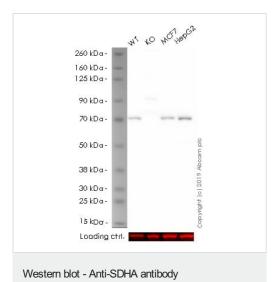
Defects in SDHA are the cause of cardiomyopathy dilated type 1GG (CMD1GG) [MIM:613642]. CMD1GG is a disorder characterized by ventricular dilation and impaired systolic function,

resulting in congestive heart failure and arrhythmia. Patients are at risk of premature death.

Sequence similarities

Belongs to the FAD-dependent oxidoreductase 2 family. FRD/SDH subfamily.

Images



[2E3GC12FB2AE2] (ab14715)

All lanes: HRP Anti-SDHA antibody [2E3GC12FB2AE2] (ab198493) at 1/5000 dilution

Lane 1 : Wild-type HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2: SDHA knockout HEK-293 whole cell lysate

Lane 3 : MCF7 (human breast adenocarcinoma cell line) whole cell lysate

Lane 4: HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

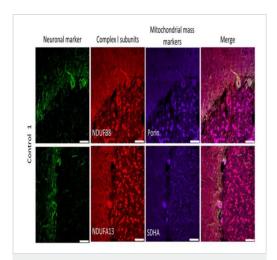
Lysates/proteins at 20 µg per lane.

Predicted band size: 70 kDa

This data was developed using the same antibody clone in a different format (HRP conjugated) (ab198493).

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab198493</u> observed at 72 kDa. Red - loading control, <u>ab181602</u>, observed at 37 kDa.

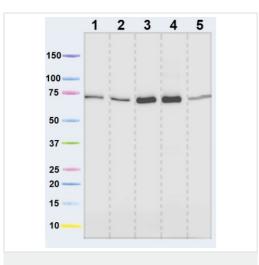
ab198493 was shown to recognize in wild-type HEK-293 cells as signal was lost at the expected MW in SDHA knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and SDHA knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab198493 and ab181602 (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Rabbit lgG H&L (IRDye® 680RD) preabsorbed ab216777 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



250 µm-thick formalin-fixed human cerebellum section were passively cleared using PACT and immunofluorescently labelled to identify mitochondrial mass (porin (ab14734, 1/100) and SDHA (ab14715, 1/100), 647 nm) and complex I subunits within the mitochondrial respiratory chain (NDUFB8 (ab110242, 1/100) and NDUFA13 (ab110240, 1/100); 546 nm) in conjunction with a neuronal marker (NF-H; 488 nm) in control 1. Scale: 100 μ m.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SDHA antibody
[2E3GC12FB2AE2] (ab14715)

Image from Phillips J et al., Sci Rep. 2016 May 16;6:26013. Fig 4a doi: 10.1038/srep26013. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/.

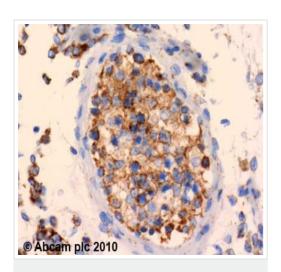


Western blot - Anti-SDHA antibody [2E3GC12FB2AE2] (ab14715)

All lanes: Anti-SDHA antibody [2E3GC12FB2AE2] (ab14715)

Lane 1: Isolated mitochondria from Human heart at 5 μg
Lane 2: Isolated mitochondria from Bovine heart at 4 μg
Lane 3: Isolated mitochondria from Rat heart at 10 μg
Lane 4: Isolated mitochondria from Mouse heart at 10 μg
Lane 5: Isolated mitochondria from HepG2 (human liver hepatocellular carcinoma cell line) at 20 μg

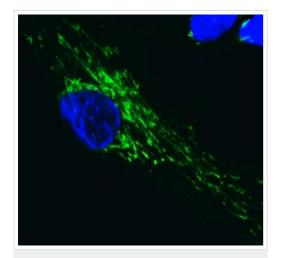
Predicted band size: 70 kDa
Observed band size: 70 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SDHA antibody
[2E3GC12FB2AE2] (ab14715)

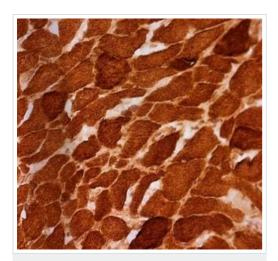
ab14715 ($2\mu g/ml$) staining SDHA in human testis using an automated system (DAKO Autostainer Plus). Using this protocol there is cytoplasmic and mitochondrial staining within the seminal vesicles.

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 peroxidase blocked in 3% H2O2 in 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 Haematoxylin 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.



Immunocytochemistry - Anti-SDHA antibody [2E3GC12FB2AE2] (ab14715)

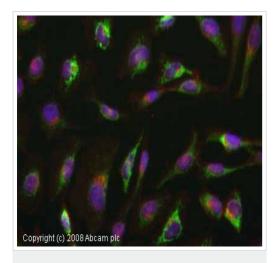
Mitochondrial localization of complex II visualized by immunocytochemistry using ab14715. Cultured human embryonic lung-derived fibroblasts (strain MRC5) were fixed, permeabilized and then labeled with ab14715 (0.2 $\mu g/ml$) followed by an AlexaFluor $^{@}$ 488-conjugated-goat-anti-mouse IgG2a isotype specific secondary antibody (2 $\mu g/ml$).



Immunohistochemistry (Frozen sections) - Anti-SDHA antibody [2E3GC12FB2AE2] (ab14715)

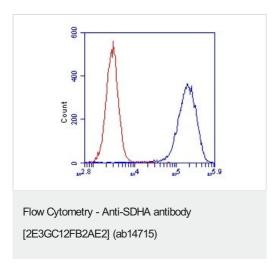
Human skeletal muscle immunohistochemistry using ab14715.

Fixed frozen tissue sections from a patient with a single large deletion of the mtDNA were used. All muscle fibers exhibit complex II immunoreactivity, consistent with the nuclear DNA-encoded expression pattern of this and all other subunits of complex II.



Immunocytochemistry - Anti-SDHA antibody [2E3GC12FB2AE2] (ab14715)

ICC/IF image of ab14715 stained human HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 0.1% PBS-tween diluted 1%BSA (OR 10% goat serum OR 0.3M glycine) for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab14715, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor[®] 488 goat anti-mouse lgG (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). This antibody also gave a positive IF result in Hek293, HepG2 and MCF7 cells.



HL-60 (human promyelocytic leukemia cell line) cells were stained with 1 μ g/mL ab14715 (blue) or an equal amount of an isotype control antibody (red) and analyzed by flow cytometry.

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