abcam

Product datasheet

Anti-PDHA1 (phospho S293) antibody ab92696

★★★★★ 11 Abreviews 67 References 7 Images

Overview

Product name Anti-PDHA1 (phospho S293) antibody

Description Rabbit polyclonal to PDHA1 (phospho S293)

Host species Rabbit

Tested applications Suitable for: WB, ICC/IF, IHC-P, ELISA

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat, Cow, Pig, Orangutan

Immunogen Synthetic peptide within Human PDHA1 aa 250-350 (phospho S293) conjugated to keyhole

limpet haemocyanin. The exact sequence is proprietary.

(Peptide available as ab104429)

Positive control This antibody gave a positive signal in Human liver tissue lysate as well as the following whole cell

lysates: HeLa; HEK293; HepG2. This antibody gave a positive result in IHC in the following FFPE

tissue: Human lung adenocarcinoma. ICC/IF: HepG2 cells.

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 pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

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Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab92696 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	****(9)	Use a concentration of 1 µg/ml. Detects a band of approximately 43 kDa (predicted molecular weight: 43 kDa).
ICC/IF	★★★★★ (2)	Use a concentration of 1 - 5 μg/ml.
IHC-P		Use a concentration of 1 µg/ml.
ELISA		Use at an assay dependent concentration.

Target

Function

The pyruvate dehydrogenase complex catalyzes the overall conversion of pyruvate to acetyl-CoA and CO(2). It contains multiple copies of three enzymatic components: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2) and lipoamide dehydrogenase (E3).

Tissue specificity

Ubiquitous.

Involvement in disease

Defects in PDHA1 are a cause of pyruvate decarboxylase E1 component deficiency (PDHE1 deficiency) [MIM:312170]. PDHE1 deficiency is the most common enzyme defect in patients with primary lactic acidosis. It is associated with variable clinical phenotypes ranging from neonatal death to prolonged survival complicated by developmental delay, seizures, ataxia, apnea, and in some cases to an X-linked form of Leigh syndrome (X-LS).

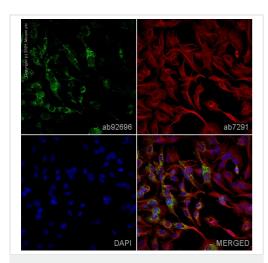
Defects in PDHA1 are the cause of X-linked Leigh syndrome (X-LS) [MIM:308930]. X-LS is an early-onset progressive neurodegenerative disorder with a characteristic neuropathology consisting of focal, bilateral lesions in one or more areas of the central nervous system, including the brainstem, thalamus, basal ganglia, cerebellum, and spinal cord. The lesions are areas of demyelination, gliosis, necrosis, spongiosis, or capillary proliferation. Clinical symptoms depend on which areas of the central nervous system are involved. The most common underlying cause is a defect in oxidative phosphorylation. LS may be a feature of a deficiency of any of the

mitochondrial respiratory chain complexes.

Cellular localization

Mitochondrion matrix.

Images



Immunocytochemistry/ Immunofluorescence - Anti-PDHA1 (phospho S293) antibody (ab92696)

1 2
260 kDa —
160 kDa —
125 kDa —
90 kDa —
70 kDa —
50 kDa —
30 kDa —
25 kDa —
25 kDa —
50 kD a —
38 kD a —
30 kD a —

Western blot - Anti-PDHA1 (phospho S293) antibody (ab92696)

ab92696 staining PDHA1 (phospho S293) in HepG2 cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-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 ab92696 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor[®] 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor[®] 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

All lanes : Anti-PDHA1 (phospho S293) antibody (ab92696) at 1 μg/ml

All lanes : HeLa Whole Cell Lysate + Calyculin A (30 nM for 20 min)

Lysates/proteins at 20 µg per lane.

Secondary

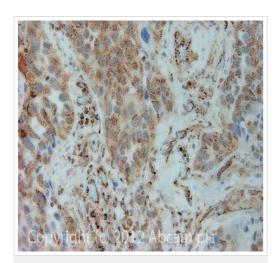
All lanes : goat anti-rabbit (green) and goat anti-mouse (red) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 43 kDa

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour and then treated with either buffer (lane 1) or alkaline phosphatase (lane 2), before being incubated with ab92696 overnight at 4°C. Antibody binding was

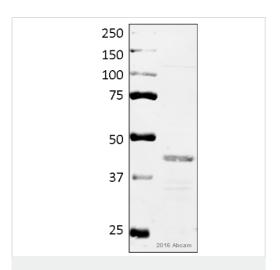
detected using IR-labelled goat anti-rabbit (green) and goat anti-mouse (Red) at 1:10,000 dilution for one hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PDHA1 (phospho S293) antibody (ab92696)

IHC image of PDHA1 (phospho S293) staining in Human lung adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab92696, 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.



Western blot - Anti-PDHA1 (phospho S293) antibody (ab92696)

This image is courtesy of an anonymous abreview.

Anti-PDHA1 (phospho S293) antibody (ab92696) at 1/1000 dilution + Human vascular smooth muscle cell whole cell lysate at 25 µg

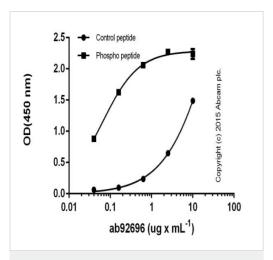
Secondary

Polyclonal goat anti-rabbit IRDye® 800CW at 1/10000 dilution

Performed under reducing conditions.

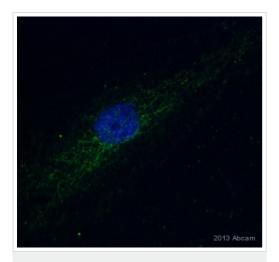
Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 5 minutes



ELISA - Anti-PDHA1 (phospho S293) antibody (ab92696)

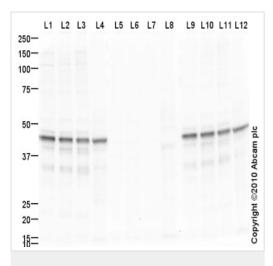
Serially diluted ab92696 was bound to immobilised Phospho peptide (S293) - or Control peptide (1 microgram x mL-1). The antibody was detected by HRP-labelled goat anti-rabbit lgG



Immunocytochemistry/ Immunofluorescence - Anti-PDHA1 (phospho S293) antibody (ab92696)

This image is courtesy of an Abreview submitted by Dimitra Kalamida

ab92696 staining PDHA1 (phospho S293) in Human HUVEC cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100 pH 7.4 for 5 minutes and blocked with 5% BSA for 20 minutes at room temperature. Samples were incubated with primary antibody (1/500 in PBS) for 1 hour . A CF488-conjugated Donkey anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.



Western blot - Anti-PDHA1 (phospho S293) antibody (ab92696)

All lanes : Anti-PDHA1 (phospho S293) antibody (ab92696) at 1 μg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

Lane 3: HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lane 4: Human liver tissue lysate - total protein (ab29889)

Lane 5 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Modified blocking peptide at 1 µg/ml

 $\mbox{\bf Lane 6: HepG2 (Human hepatocellular liver carcinoma cell line)} \label{eq:line} Whole Cell Lysate with Modified blocking peptide at 1 $\mu g/ml$ }$

Lane 7 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate with Modified blocking peptide at 1 μ g/ml

Lane 8 : Human liver tissue lysate - total protein (<u>ab29889</u>) with Modified blocking peptide at 1 μ g/ml

Lane 9: HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Non-modified blocking peptide at 1 µg/ml

Lane 10 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate with Non-modified blocking peptide at 1 μ g/ml

Lane 11 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate with Non-modified blocking peptide at 1 µg/ml

Lane 12 : Human liver tissue lysate - total protein ($\underline{ab29889}$) with Non-modified blocking peptide at 1 μ g/ml

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 43 kDa **Observed band size:** 43 kDa

Additional bands at: 34 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes

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

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