

Product datasheet

Phospho S300 PDH E1 alpha protein (PDHA1) Profiling ELISA Kit ab115345

[1 References](#) [3 Images](#)

Overview

Product name Phospho S300 PDH E1 alpha protein (PDHA1) Profiling ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
1	8			2.5%

Inter-assay

Sample	n	Mean	SD	CV%
1	4			7.8%

Sample type Cell culture extracts, Tissue Extracts

Assay type Sandwich (quantitative)

Sensitivity 15 µg/ml

Range 15 µg/ml - 500 µg/ml

Assay duration Multiple steps standard assay

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

Product overview ab115345 is an in vitro enzyme-linked immunosorbent assay to determine the levels of phospho S300 PDHA1 protein in cell and tissue lysates. The assay employs a mouse antibody specific for PDHA1 protein coated on a 96-well plate. Samples are pipetted into the wells and PDHA1 protein present in the sample is bound to the wells by the immobilized antibody. The wells are washed and a rabbit anti-phospho S300 PDHA1 protein detector antibody is added. After washing away unbound detector antibody, HRP-conjugated anti-rabbit antibody is pipetted into the wells. The wells are again washed, an HRP substrate solution (TMB) is added to the wells and color develops in proportion to the amount of phospho S300 PDHA1 protein bound. The developing blue color is measured at 600 nm. Optionally the reaction can be stopped by adding hydrochloric acid which changes the color from blue to yellow and the intensity can be measured at 450 nm.

Notes Store all components at 4°C. This kit is stable for 6 months from

receipt. Unused microplate strips should be returned to the pouch containing the desiccant and resealed.

Platform Microplate

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Blocking Buffer	1 x 6ml
10X HRP Label	1 x 1ml
10X phospho S300 PDHA1 protein Detector Antibody	1 x 0.7ml
20X Buffer	1 x 20ml
Extraction Buffer (ab260490)	1 x 15ml
Microplate 96 antibody coated wells in 12 strips	1 unit
HRP Development Solution	1 x 12ml

Function The pyruvate dehydrogenase complex catalyzes the overall conversion of pyruvate to acetyl-CoA and CO₂. It contains multiple copies of three enzymatic components: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2) and lipoamide dehydrogenase (E3).

Tissue specificity Ubiquitous.

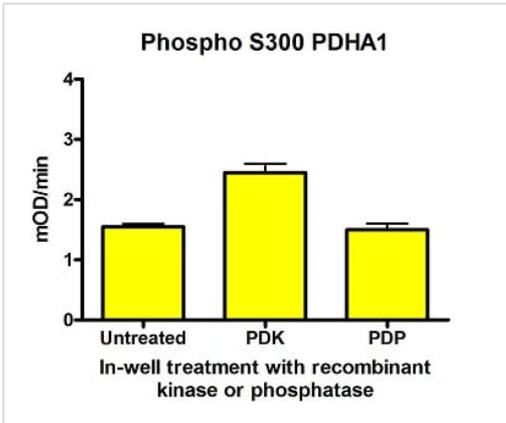
Involvement in disease Defects in PDHA1 are a cause of pyruvate dehydrogenase E1-alpha deficiency (PDHAD) [MIM:312170]. An enzymatic defect causing primary lactic acidosis in children. It is associated with a broad clinical spectrum ranging from fatal lactic acidosis in the newborn to chronic neurologic dysfunction with structural abnormalities in the central nervous system without systemic acidosis.

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.

Post-translational modifications Phosphorylation at Ser-293 by PDK family kinases blocks the access to active site, and inactivates the enzyme.

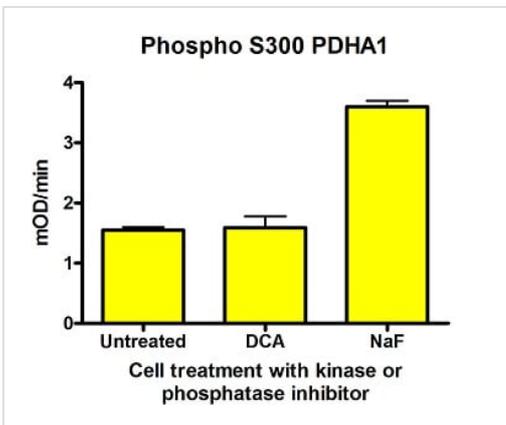
Cellular localization Mitochondrion matrix.

Images



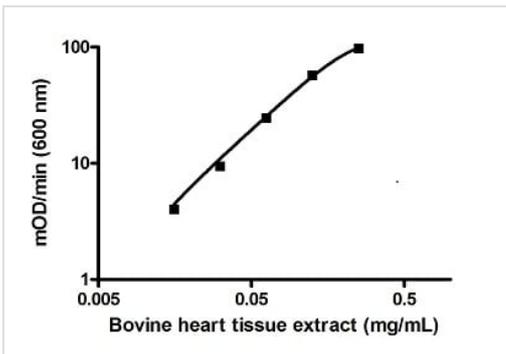
- Phospho S300 PDH E1 alpha protein (PDHA1)
ELISA Kit (ab115345)

The PDHA1 bound from undosed HeLa cells was subject to in-well kinase treatment (PDK1&3) or in-well phosphatase treatment (PDP1) according to the supplementary protocol shown below. Untreated cells did not show a significant endogenous phosphorylation signal at S300 this could be increased by kinase treatment. Phosphatase treatment had no effect on the endogenous (low) level of phospho S300 signal.



- Phospho S300 PDH E1 alpha protein (PDHA1)
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HeLa cells were cultured for 4 hours in media supplemented with DCA (20mM) to specifically inhibit mitochondrial PDH kinases, or NaF (20mM), a general inhibitor of serine/threonine protein phosphatases. The DCA treatment did not reduce the level of phospho S300 confirming that there is little endogenous phospho S300. Conversely NaF treatment, to inhibit cellular serine phosphatases, did increase the phosphorylation level of S300.



ELISA - Phospho S300 PDH E1 alpha protein (PDHA1) ELISA Kit (ab115345)

The phosphorylation state of PDHA1 can vary by treatment but also by cell culture conditions such as media supplements, nutrients and also cell density.

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