Product datasheet

Anti-Pyruvate Dehydrogenase E1-alpha subunit antibody [EPR11098] ab168379

Overview

Product name Anti-Pyruvate Dehydrogenase E1-alpha subunit antibody [EPR11098]
Description Rabbit monoclonal [EPR11098] to Pyruvate Dehydrogenase E1-alpha subunit
Host species Rabbit
Tested applications Suitable for: WB, Flow Cyt, ICC/IF, IHC-P, IP
Species reactivity Reacts with: Mouse, Rat, Human
Immunogen Synthetic peptide within Human Pyruvate Dehydrogenase E1-alpha subunit aa 100-200. The exact sequence is proprietary.
(Peptide available as ab170730)
Positive control Human fetal kidney, A549, Jurkat, HepG2 and HeLa lysates; Human kidney and skeletal muscle tissues; HepG2 cells; permeabilized Jurkat cells; HT-29; Mouse kidney; Rat kidney.
General notes Our RabMAB® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAB® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated ‘PUR’ on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties

Form Liquid
Storage buffer Preservative: 0.01% Sodium azide
Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity Protein A purified
Clonality Monoclonal
Clone number: EPR11098  
Isotype: IgG

**Applications**

Our Abpromise guarantee covers the use of ab168379 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>1/10 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>ICC/IF</td>
<td></td>
<td>1/100 - 1/500.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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<td>IP</td>
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<td>1/10 - 1/100.</td>
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**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**

2
Western blot - Anti-Pyruvate Dehydrogenase E1-alpha subunit antibody [EPR11098] (ab168379)

All lanes : Anti-Pyruvate Dehydrogenase E1-alpha subunit antibody [EPR11098] (ab168379) at 1/1000 dilution

Lane 1 : Wild-type HeLa whole cell lysate
Lane 2 : PDHA1 knockout HeLa whole cell lysate
Lane 3 : HEK-293 whole cell lysate

Lysates/proteins at 20 µg per lane.

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

Lanes 1 - 3: Merged signal (red and green). Green - ab168379 observed at 43 kDa. Red - loading control, ab130007, observed at 130 kDa.

ab168379 was shown to recognize PDHA1 in wild-type HeLa cells as signal was lost at the expected MW in PDHA1 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and PDHA1 knockout samples were subjected to SDS-PAGE. Ab168379 and ab130007 (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-Pyruvate Dehydrogenase E1-alpha subunit antibody [EPR11098] (ab168379) at 1/2000 dilution

Lane 1 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates
Lane 2 : Mouse brain lysates
Lane 3 : Rat brain lysates
Lane 4 : Mouse kidney lysates
Lane 5 : Rat kidney lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000
**Predicted band size:** 43 kDa  
**Observed band size:** 43 kDa

Blocking and diluting buffer: 5% NFDM/TBST

Immunohistochemistry/Immunofluorescence analysis of HT-29 (Human colorectal adenocarcinoma epithelial cell) cells labeling Pyruvate Dehydrogenase E1-alpha subunit with Purified ab168379 at 1:100 dilution (3.5μg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 μg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Immunoprecipitation - Anti-Pyruvate Dehydrogenase E1-alpha subunit antibody [EPR11098] (ab168379)  
ab168379 (purified) at 1:20 dilution (2ug) immunoprecipitating Pyruvate Dehydrogenase E1-alpha subunit in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate.  
**Lane 1 (input):** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10ug  
**Lane 2 (+):** ab168379 & HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate  
**Lane 3 (-):** Rabbit monoclonal IgG (ab172730) instead of ab168379 in HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate  
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.  
Blocking and diluting buffer: 5% NFDM/TBST.
Flow Cytometry analysis of Jurkat (Human T cell leukemia T lymphocyte) cells labeling Pyruvate Dehydrogenase E1- alpha subunit with purified ab168379 at 1:40 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilized with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue sections labeling Pyruvate Dehydrogenase E1-alpha subunit with Purified ab168379 at 1:200 dilution (1.76 μg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue sections labeling Pyruvate Dehydrogenase E1-alpha subunit with Purified ab168379 at 1:200 dilution (1.76 μg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue sections labeling Pyruvate Dehydrogenase E1-alpha subunit with Purified ab168379 at 1:200 dilution (1.76 μg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, pH9.0. Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.
Immunocytochemistry/Immunofluorescence analysis Jurkat (human acute T cell leukemia) labelling Pyruvate Dehydrogenase E1-alpha subunit with purified ab168379 at 1/500. Cells were fixed with 100% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Control: PBS only

All lanes: Anti-Pyruvate Dehydrogenase E1-alpha subunit antibody [EPR11098] (ab168379) at 1/1000 dilution (unpurified)

Lane 1: Human fetal kidney lysate
Lane 2: A549 lysate
Lane 3: Jurkat lysate
Lane 4: HepG2 lysate
Lane 5: HeLa lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 43 kDa
Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling Pyruvate Dehydrogenase E1-alpha subunit with unpurified ab168379 at 1/100 dilution.

Immunohistochemical analysis of paraffin-embedded Human skeletal muscle tissue labeling Pyruvate Dehydrogenase E1-alpha subunit with unpurified ab168379 at 1/100 dilution.

Immunofluorescent analysis of HepG2 cells labeling Pyruvate Dehydrogenase E1-alpha subunit with unpurified ab168379 at 1/100 dilution.
Flow cytometric analysis of permeabilized Jurkat cells labeling Pyruvate Dehydrogenase E1-alpha subunit (red) with unpurified ab168379 at 1/10 dilution, or a rabbit IgG (negative) (green).

Detection of Pyruvate Dehydrogenase E1-alpha subunit by Western Blot of Immunprecipitate. 293T cell lysate immunoprecipitated using unpurified ab168379 at 1/10 dilution; HRP-conjugated anti-rabbit IgG preferentially detecting the non-reduced form of rabbit IgG.

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