PDH application guide

Pyruvate Dehydrogenase Complex

Tips and techniques for researching PDH using MitoSciences research tools

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Introduction

The pyruvate dehydrogenase complex (PDH or PDC) is a key regulatory site in cellular metabolism, by linking the citric acid cycle and subsequent oxidative phosphorylation with glycolysis and gluconeogenesis, as well as with both lipid and amino acid metabolism. When carbohydrate stores are reduced in mammals, PDH activity is regulated downward to limit the use of glucose by oxidative phosphorylation in tissues that can use fatty acids or ketone bodies, such as heart and skeletal muscle. The important exception is neuronal tissue, which processes glucose almost exclusively for ATP production.

Activation of PDH both facilitates use of carbohydrate to meet energy demands and also converts surplus dietary carbohydrates to fatty acids for longer term energy storage. Perturbation of the regulation of this choice of glucose or fatty acids as energy source is a key part of diabetes, metabolic syndrome and obesity, while metabolic substrate switching from oxidative phosphorylation to glycolysis defines the cancer phenotype, hence recent renewed interest in PDH activity and regulation.

Structure and Activity

The pyruvate dehydrogenase complex is a 9.5 megadalton assembly of four proteins: pyruvate dehydrogenase (E₁), dihydrolipoamide acyltransferase (E₂), dihydrolipoyl dehydrogenase (E₃), and one structural protein (E₂/E₃ binding protein). The E₁ enzyme is a heterotetramer of two α and two β subunits. PDH component proteins are arranged as a core of 60 E₂ subunits around which are distributed 30 copies of E₁, 12 copies of E₃, and 12 copies of the E₂/E₃ binding protein. PDH catalyzes irreversible oxidative decarboxylation of pyruvate to acetyl Coenzyme A, as shown in Figure 1.

![Figure 1](image.png) **Figure 1.** Five sequential reactions of PDH-catalyzed oxidative decarboxylation of pyruvate to Acetyl Coenzyme A.
Regulation of PDH Activity by Phosphorylation

Not surprisingly given its central role in metabolism, PDH is under tight and complex regulation, which includes regulation by reversible phosphorylation in response to the availability of glucose. In humans, PDH activity is inhibited by site-specific phosphorylation at three sites on the E1α subunit (Ser232, Ser293 and Ser300), which is catalyzed by four different pyruvate dehydrogenase kinases (PDK1-4). Each of the four kinases has a different reactivity for these three sites. Interestingly, phosphorylation at any one site leads to the inhibition of the complex in vitro. Two pyruvate dehydrogenase phosphatases (PDP1 and PDP2) dephosphorylate the E1α and activate the enzyme. The phosphatases show little or no site specificity. Both the kinases and phosphatases are differentially expressed in tissues. Each of the PDK’s and PDP’s is under transcriptional control in response to different cellular stress events as shown in Figure 2. In addition, the kinases are activated by acetyl coenzyme A, NADH and ATP, meanwhile the availability of pyruvate and ADP leads to their inhibition.

**Figure 2.** A schematic of the reactions controlling pyruvate dehydrogenase.

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As an example of the transcriptional regulation, expression of PDK4 is suppressed under basal conditions in most tissues by maintaining relevant histones in a deacetylated state, but its expression is increased during starvation by glucocorticoids that re-acetylate these histones, particularly in heart, skeletal and other muscle tissues, kidney, and liver. PDK4 is also up-regulated by a high fat diet and extended exercise. Insulin inhibits PDK4 expression via PI3K signaling that leads to lower histone acetylation. The levels of PDK4 are also regulated to PPAR transcription factors. Importantly, in diabetes caused by either insulin deficiency or insulin insensitivity, the uninhibited PDK4 over-expression prevents glucose oxidation.

In contrast, the levels of PDK1 are sensitive to O_2 levels and under regulation by the transcription factor HIF-1α. An increase in the level of PDK1 is a key part of the so-called Warburg effect, a switch from oxidative to glycolytic ATP production that characterizes cancer cells.

**Research tools for PDH Analysis**

Our antibodies have been rigorously validated for target specificity by a combination of immunoprecipitation, Western blot, immunocytochemistry (ICC) and ELISA assays. Antibodies validated in flow cytometry are immunocytochemistry positive. All available flow-validated antibodies against PDH subunits have a minimum 5-fold greater staining intensity than the appropriate isotype control.

MitoSciences research tools provide a convenient way to assay PDH activity in complex biological samples, by isolating the enzyme in its active form and in complexes that likely represent their associations in situ. The active enzyme can be separated by immunocapture onto a solid surface in two ways: 96-well microplate format (ab109902, ab110671) or dipstick assay (ab109882). NADH production can then be measured directly without interference from other NADH-utilizing or producing enzymes. This approach is simpler, faster and safer than the classical method of using [14C] pyruvate and measuring enzyme-catalyzed release of [14C] CO_2.

Active PDH regulatory enzymes are available: four PDH kinases (PDK 1-4: ab110353, ab110354, ab110355, ab110365) and both PDH phosphatases (PDP 1&2: ab110358, ab110359).

For convenience, detailed optimized protocols are provided to carry out the phosphorylation and dephosphorylation on immunocaptured PDH followed by PDH activity or phospho-serine detection using PDH E1α phospho S232, S293 and S300 ELISA kits (ab115343, ab115344, ab115345).
Figure 3. Dipstick enzyme activity assays apply a novel approach by utilizing lateral flow concept, where capture antibodies are striped onto nitrocellulose membrane and a wicking pad draws the sample through the antibody bands (ab109882).
I. Assaying the Endogenous and Phosphorylated PDH Activity in Extracts

The principle regulator of glucose metabolism is the activity of PDH which can be measured after isolation from tissues and cells. The key to such measurements is maintaining the enzyme in the *in vivo* state of phosphorylation throughout the isolation process. Extraction and immunocapture buffers formulated to inhibit endogenous specific and non-specific kinases and phosphatases prevent unwanted PDH modifications during the sample preparation (see Figure 4). Protocols are available for using human, bovine, rat and mouse cell or tissue extracts.

Following immunocapture of PDH and removal of endogenous kinases and phosphatases, parallel samples can be phosphorylated with recombinant PDK’s (ab110353, ab110354, ab110355, ab110365) and dephosphorylated with recombinant PDP’s (ab110358, ab110359) to respectively determine (a) residual activity of the fully-phosphorylated PDH, (b) maximum activity of the fully dephosphorylated enzyme and (c) endogenous unmodified PDH activity.

![Figure 4. PDH kinases have no effect on PDH activity in the absence of ATP (green) or presence ATP+apyrase (blue). However kinases did inhibit activity when ATP was added (red), subsequent treatment with apyrase (yellow) had no effect. Activity was unaffected in the absence of kinase.](image)
II. Assaying PDH Quantity and Phosphorylation State in Extracts

As mentioned above, the key regulatory subunit of PDH is the E1α subunit (PDHA1), which is modified by kinases at three serine phosphorylation sites to decrease activity. As part of the MitoSciences PDH range are 96-well microplate sandwich ELISA assays to measure each of the modifications phospho S232 (ab115343), phospho S293 (ab115344), phospho S300 (ab115345) and total PDH E1α (ab115342). These measurements can be performed to identify the endogenous levels of protein and phosphorylation, or their levels as a result of treatments to cells or tissues (see Figure 5).

![ELISA Assay Graphs](image)

**Figure 5.** Using these ELISA kits, lysates from HeLa cells cultured in the PDH kinase inhibitor dichloroacetate (DCA, 20mM) show a significant decrease in phosphorylation at all three regulatory serines in PDH E1α. Conversely using the non-specific serine phosphatase inhibitor sodium fluoride (NaF, 10 mM), phosphorylation at each site increases. Neither treatment significantly affected the total expression level of total PDH E1α subunit. Interestingly, in untreated cells, there is an endogenous phosphorylation level at serine 232 and 293 indicating a large quantity of inactive enzyme. Conversely the endogenous phosphorylation state of serine 300 is low.

Additionally using PDH kinases 1-4 (ab110359, ab110354, ab110355,ab110356) and phosphatases 1&2 (ab110359,ab110358), maximal and minimal phosphorylation states can be achieved by phosphorylation or dephosphorylation reactions in each well after the target is captured and all other cellular components are removed. Use of these enzymes in conjunction with each ELISA is described in the protocols for each assay.
III. Cell-Based Immunofluorescent Assays for PDH analysis

In-Cell ELISA (ICE) uses quantitative immunocytochemistry to measure protein levels or post-translational modifications in cultured cells in a high-throughput format. The benefit of the ICE method is that rapid fixation of cells in a 96- or 384-well plate in situ stabilizes the phosphorylated residues of the enzyme and eliminates any changes during sample preparation. Target(s) of interest are detected with highly specific, well characterized antibodies, followed by labeled secondary antibodies.

Phosphorylation of PDH E1α using ICE

Detection of phosphorylated PDH E1α with fluorescent IRDyes® requires a LI-COR® Odyssey® or Aerius® imaging system to produce raw signals with greater sensitivity and reproducibility (ab110216). An alternative is a colorimetric version and is compatible with standard plate readers (ab110217). Typically cells analyzed are adherent to the cell culture plate, however a protocol for suspension cells is available (ab111542). Phosphorylation of PDH E1α at each of the regulatory serine residues (232, 293 and 300) are measured simultaneously with measure the total PDH E1α protein level in each well, allowing simple signal normalization. The utility of the assay is demonstrated on cells treated with dichloroacetate, DCA, in Figure 6.

![Graphs and images showing the effect of DCA on PDH kinase dependent phosphorylation of PDH E1α S232, S293 and S300 in different cell lines.](image)

Figure 6. Dichloroacetate (DCA) is a known inhibitor of PDH kinases. Using PhosphoPDH In-Cell ELISA Kit (IR) (ab110218) the effect of DCA on the PDH kinase dependant phosphorylation of PDH E1α S232, S293 and S300 is shown in three different human cell lines HeLa, HDFn (fibroblast) and HepG2.
Transcriptional regulation of PDK1 by Hif1 in hypoxia

In-Cell ELISA (ICE) assay ab125299 uses quantitative immunocytochemistry to measure HIF1 α and PDK1 protein levels in cultured cells. It is also available as a flow cytometry antibody cocktail (ab126541).

Stabilization of the HIF1 α transcription factor directly leads to increased transcription of PDK1. The PDH complex transforms pyruvate to acetyl coA for use in the TCA cycle. Hence inhibitory phosphorylation of PDH by PDK1 results in down regulation of TCA function and oxidative phosphorylation.

Figure 7. HeLa cells were treated with deferoxamine (DFO) to simulate hypoxic conditions. (A) At concentrations of 10 μM and higher, Hif1α is stabilized leading to (B) transcription of proteins in the Hif hypoxia pathway which control aerobic glycolysis including PDH Kinase 1 (PDK1).
IV. Individual Antibodies for Detection of PDH subunits by ICC, ICE, Flow and Western blot

Defects in the PDH complex are an important cause of lactic acidosis and can cause Leigh’s Diseases in children. Mutations of E2, E3 and E2 /E3 binding proteins have been reported, but the majority of cases of PDH deficiency are due to mutations in the X-linked E1α subunit. Female carriers of E1α are present mostly as mosaics, often with only a small percentage of defective cells. PDH E1α can be screened using ab110330 by immunocytochemistry of patient cell lines or tissue as shown in Figure 8.

**Figure 8.** Immunocytochemical analyses of a PDH complex assembly defect in a patient derived cell line which has a PDH E1a subunit mutation. Antibodies used to detect representative subunits for all 5 OXPHOS complexes (Complex I ab109798, Complex II ab14714, Complex III ab14745, Complex IV ab14705, Complex V ab14748) and E1a of PDH (ab110330) are shown by green staining. Counter staining with VDAC/porin (ab14734) is shown in red, demonstrating loss of E1 α signal in this cell line where OXPHOS complexes are unaffected.
**Pyruvate dehydrogenase (PDH) immunocapture monoclonal antibody (ab109866)**

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<th>Applications</th>
<th>Amount</th>
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<tbody>
<tr>
<td>H, B</td>
<td>IP</td>
<td>100 µg</td>
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MSP01c is an E2 binding antibody capable of immunoprecipitating the entire PDH complex when following MitoSciences’ immunoprecipitation protocol. This antibody is also available in a ready-to-use immunocapture kit, containing the antibody crosslinked to agarose beads (MSP01).

**Pyruvate dehydrogenase (PDH) subunit E1α monoclonal antibody (ab110330)**

<table>
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<tr>
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<th>Applications</th>
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<td>H, B, M</td>
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**UniProt Number:** P08559

**Alternate Names:** Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial, PDHE1-A type I

**Structure and Function:** The pyruvate dehydrogenase complex contains three different subunits including the pyruvate dehydrogenase E1 subunit. The PDH complex is responsible for catalyzing the formation of acetyl-CoA and CO2 from pyruvate.

**Disease Associations:** Enzyme deficiency of the PDH E1 subunit of the PDH complex commonly results in lactic acidosis. There are a wide range of symptoms that characterize this disorder including neonatal death, developmental delay, seizures, ataxia, apnea and X-link Leigh Syndrome (LS). This syndrome presents itself with necrotic lesions that are located symmetrically in both sides the subcortical brain.
Pyruvate dehydrogenase (PDH) subunit E1α monoclonal antibody (ab110334)

**Reactivity**
H, B, M, R, Ce, Dr

**Applications**
WB, ICC, ICE, Flow

**Amount**
100 µg

**UniProt Number:** P08559

**Alternate Names:** Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial, PDHE1-A type I

**Structure and Function:** The pyruvate dehydrogenase complex contains three different subunits including the pyruvate dehydrogenase E1 subunit. The PDH complex is responsible for catalyzing the formation of acetyl-CoA and CO2 from pyruvate.

**Disease Associations:** Enzyme deficiency of the PDH E1 subunit of the PDH complex commonly results in lactic acidosis. There are a wide range of symptoms that characterize this disorder including neonatal death, developmental delay, seizures, ataxia, apnea and X-link Leigh Syndrome (LS). This syndrome presents itself with necrotic lesions that are located symmetrically in both sides the subcortical brain.

Pyruvate dehydrogenase (PDH) subunit E1 beta monoclonal antibody (ab110331)

**Reactivity**
H, B, M, R

**Applications**
WB

**Amount**
100 µg

**UniProt Number:** P11177

**Alternate Names:** Pyruvate dehydrogenase E1 component subunit beta, mitochondrial, PDHE1-B

**Structure and Function:** The pyruvate dehydrogenase complex contains three different subunits including the pyruvate dehydrogenase E1 subunit. The PDH complex is responsible for catalyzing the formation of acetyl-CoA and CO2 from pyruvate.

**Disease Associations:** Enzyme deficiency of the PDH E1 subunit of the PDH complex commonly results in lactic acidosis. There are a wide range of symptoms that characterize this disorder including neonatal death, developmental delay, seizures, ataxia, apnea and X-link Leigh Syndrome (LS). This syndrome presents itself with necrotic lesions that are located symmetrically in both sides the subcortical brain.
**Pyruvate dehydrogenase (PDH) subunit E2 monoclonal antibody (ab110332)**

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**UniProt Number:** P10515

**Alternate Names:** Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial, M2 antigen complex 70 kDa subunit, Pyruvate dehydrogenase complex E2 subunit, PDC-E2, PDCE2, E2, Dihydrolipoamide S-acetyltransferase component of pyruvate dehydrogenase complex 70 kDa mitochondrial autoantigen of primary biliary cirrhosis, PBC

**Structure and Function:** The pyruvate dehydrogenase complex contains three different subunits including the dihydrolipoamide acetyltransferase (E2) subunit.

**Disease Associations:** The E2 subunit of the PHD complex is associated with primary biliary cirrhosis in that patients diagnosed with this disease have autoantibodies for this subunit. This condition causes damage to liver cells and can result in cirrhosis of the liver. Lactic acidemia (PDH deficiency) is another condition associated with the E2 subunit. The clinical characterization of this disorder includes primary lactic acidosis as well as neurological problems, mainly episodic dystonia.

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**Pyruvate dehydrogenase (PDH) subunit E2/E3bp monoclonal antibody (ab110333)**

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**UniProt Number:** Subunit (E2): P10515; Subunit (E3bp): O00330

**Alternate Names:** Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial, Pyruvate dehydrogenase (Lipoamide) alpha 1, Pyruvate dehydrogenase complex E2 subunit, PDC-E2, PDCE2, E2, Dihydrolipoamide S-acetyltransferase component of pyruvate dehydrogenase complex 70 kDa mitochondrial autoantigen of primary biliary cirrhosis, PBC, M2 antigen complex 70 kDa subunit

**Structure and Function:** The pyruvate dehydrogenase complex contains three different subunits including the dihydrolipoamide acetyltransferase (E2) subunit as well as the lipoamide dehydrogenase (E3) subunit.

**Disease Associations:** The E2 subunit of the PHD complex is associated with primary biliary cirrhosis in that patients diagnosed with this disease have autoantibodies for this subunit. This condition causes damage to liver cells and can result in cirrhosis of the liver. Lactic acidemia (PDH deficiency) is another condition associated with the E2 subunit. The clinical characterization of this disorder includes primary lactic acidosis as well as neurological problems, mainly episodic dystonia.
**Pyruvate dehydrogenase kinase isoform 1 (PDK1) monoclonal antibody (ab110335)**

**Reactivity** | **Applications** | **Amount**
--- | --- | ---
H, B, M, R | WB, ICC, ICE, Flow | 100 µg

**UniProt Number:** Q15118  
**Alternate Names:** Pyruvate dehydrogenase [lipoamide] kinase isozyme 1, PDHK1  
**Structure and Function:** Pyruvate dehydrogenase kinase 1 (PDK1) inhibits the mitochondrial pyruvate dehydrogenase complex by phosphorylation of the E1α subunit, thus contributing to the regulation of glucose metabolism. Hypoxia can directly induce PDK1 expression via Hif1α mediate pathway. Increased expression results in inactivation of PDH and the TCA cycle and subsequent suppression of metabolism. PDK1 enzyme activity regulated by dichloracetate, radicicol and AZD7545.  
**Disease Associations:** Cancer metabolism, ischaemic diseases

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**Pyruvate dehydrogenase kinase isoform 4 (PDK4) monoclonal antibody (ab110336)**

**Reactivity** | **Applications** | **Amount**
--- | --- | ---
H | WB | 100 µg

**UniProt Number:** Q16654  
**Alternate Names:** Pyruvate dehydrogenase [lipoamide] kinase isozyme 4, PDHK4  
**Structure and Function:** PDK4 is an enzyme that in humans is encoded by the PDK4 gene. It codes for an isozyme of pyruvate dehydrogenase kinase. This protein is located in the matrix of the mitochondria and inhibits the mitochondrial pyruvate dehydrogenase complex by phosphorylation of the E1α subunit, reducing the conversion of pyruvate, which is produced from the oxidation of glucose and amino acids, to acetyl-CoA and contributing to the regulation of glucose metabolism. Expression of this gene is regulated by glucocorticoids, retinoic acid and insulin. PDK4 is increased in hibernation and helps to decrease metabolism and conserve glucose by decreasing its conversion to acetyl-CoA, which enters the citric acid cycle and is converted to ATP.  
**Disease Associations:** Cancer metabolism, ischaemic diseases. PDK4 is selectively upregulated in the longer term in most tissues and organs in response to starvation and hormonal imbalances such as insulin resistance, diabetes mellitus and hyperthyroidism.
Kits and assays for PDH

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<td>Pyruvate dehydrogenase (PDH) Enzyme Activity Dipstick Assay Kit</td>
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PDH Proteins and enzymes

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Ready-to-use kits and detailed protocols to investigate PDH as described in this PDH Playbook can be found at the following links:

- **MitoSciences PDH product page:**
  www.abcam.com/PDH-Product-range

- **PDH Protocols:** www.abcam.com/PDH-Protocols
  (This web page includes protocols for high-throughput screening investigation of the phosphorylation status and phosphorylation-regulated activity of PDH.)

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