ab110174 – Pyruvate dehydrogenase (PDH) Profiling ELISA Kit

Instructions for Use

For the measurement of Pyruvate dehydrogenase (PDH) in Human, bovine, mouse, and rat whole tissue or cell lysate samples.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Pyruvate dehydrogenase (PDH) *in vitro* Profiling ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the measurement of Pyruvate dehydrogenase (PDH) in Human, bovine, mouse, and rat whole tissue or cell lysate samples.

Capture antibodies are pre-coated in the wells of modular microplates, which can be broken into 8-well strips. This assay is a "sandwich" ELISA, where the PDH enzyme is purified and immobilized by an anti-PDH capture antibody pre-coated in the microplate wells. The amount of captured PDH is determined by adding a second (detector) anti-PDH antibody which binds to the captured PDH hat a different epitope. This is followed by binding of an HRP conjugated goat anti-mouse antibody that binds the detector anti-PDH antibody. The detector-bound HRP then changes the colorless HRP development solution to blue and the color intensity (absorbance) is proportional to the amount of PDH captured. All of our microplate assays utilize our highly-validated immunocapture antibodies, which are able to capture large, multi-subunit enzyme complexes in their fully intact state.

PDH is the key regulatory enzyme of cellular metabolism because it links the TCA cycle and subsequent oxidative phosphorylation with glycolysis and gluconeogenesis as well as with both lipid and amino acid metabolism. PDH activity is regulated primarily by PDK-dependent phosphorylation and PDP-dependent dephosphorylation of PDH. Phosphorylation inactivates PDH whereas dephosphorylation activates PDH. Phosphorylation occurs at Serines 232, 293, and 300 of the human E1α subunits.

This kit can also be used as the basis for additional sandwich assays using alternative detector monoclonal antibodies (not provided) specific for certain phospho-serine residues on PDH that are reversibly phosphorylated/dephosphorylated to modify PDH activity in response to metabolic demands e.g.

Phospho-PDH Ser293 (Site 1) polyclonal antibody

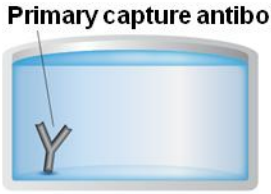
Phospho-PDH Ser300 (Site 2) polyclonal antibody

Phospho-PDH Ser232 (Site 3) polyclonal antibody

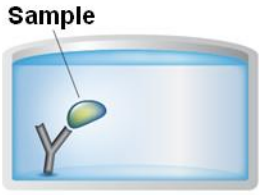
Abcam also offers a comprehensive line of PDH-related assays and reagents that can be used in conjunction with this kit to elucidate various aspects of PDH activity, physiologic regulation and phosphorylation status. These include all four PDH kinases, both PDH phosphatases, PDH activity microplate assays and PDH protein quantity microplate assays. For convenience, these tools are available combined in several kits and described in additional protocols.

The three alternative phospho-serine detector antibodies listed above can be employed easily with this kit simply by replacing the “PDH detector mAb” with one of the Phospho-PDH Serine specific antibodies in wells selected for phospho-site detection. Because the phospho-site specific antibodies are of rabbit origin, and not mouse, it is also necessary to replace the HRP-goat-anti-mouse secondary antibody normally employed with an appropriate HRP-goat-anti-rabbit antibody in each well selected for phospho-site detection. As noted in the “Sample Preparation” section, particular care must be taken to preserve the endogenous phosphorylation state during sample preparation when using the phospho-site-specific alternative detector antibodies.

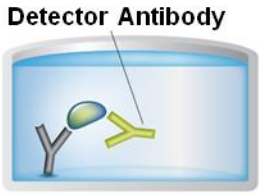
2. ASSAY SUMMARY



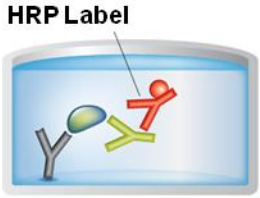
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and controls as instructed.



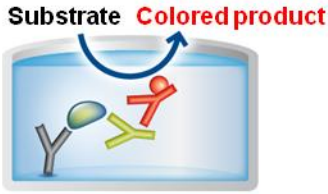
Add control or sample to each well used. Incubate at room temperature.



Aspirate and wash each well. Add prepared Detector Antibody to each well. Incubate at room temperature.



Aspirate and wash each well. Add prepared HRP label. Incubate at room temperature.



Aspirate and wash each well. Add HRP Development Solution to each well. Immediately begin recording the color development. Alternatively add a stop solution at a user-defined time.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +2-8°C immediately upon receipt, except 5X Stabilizer which should be stored at -80°C

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent and Sample Preparation sections.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
20X Buffer	20 mL	+2-8°C
10X Blocking Buffer	10 mL	+2-8°C
1X HRP Development Solution	20 mL	+2-8°C
Detergent	1 mL	+2-8°C
20X Detector Antibody	1 mL	+2-8°C
20X HRP Label	1 mL	+2-8°C
96 – Well microplate (12 x 8 well strips)	96 wells	+2-8°C
5X Stabilizer	13 mL	-80°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Spectrophotometer that measures absorbance at 600nm
- Multichannel pipette (50 - 300 µL) and tips
- Protein assay method (e.g BCA)
- Phosphate buffered saline (PBS)
- Optional for 450 nm endpoint data measurement – 1 N HCl
- Deionized water

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Samples generating values higher than the highest control should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, control and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 10).
- All samples should be mixed thoroughly and gently.
- Avoid multiply freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps (optional).
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample or control will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1 **Incubation Solution**

Prepare Incubation Solution by mixing 1 part 10X Blocking Buffer with 9 parts 1X Buffer (the total volume of Incubation Solution needed per experiment depends on the number of wells to be used in the experiment at hand).

9.2 **1X Buffer**

Prepare 1X Buffer by mixing 15 mL of 20X Buffer to 285 mL deionized H_2O.

9.3 **1X Detector Antibody**

Prepare the 1X Detector Antibody by mixing 1 part 20X Detector Antibody with 19 parts Incubation Solution.

9.4 **1X Stabilizer**

Prepare 1X Stabilizer by mixing 1 part 5X Stabilizer with 4 parts 1X Buffer.

9.5 **1X HRP Label**

Prepare 1X HRP label by mixing 1 part 20X HRP Label with 19 parts Incubation Solution.

10. SAMPLE PREPARATION

The protein concentration of the sample should be measured before solubilization. Once diluted to the specified concentration the sample is detergent-solubilized and diluted to within the linear range of measurement. A control or normal sample should always be included in the assay as a reference positive control measurement. In addition, a buffer control should be used as a negative control.

NOTE: If phospho-serine detector antibodies are used in place of the standard PDH detector mAb, it is critical to inhibit the endogenous PDH phosphatases and kinases during sample preparation and immunocapture to ensure the phosphorylation status of the sample does not change during processing.

10.1 Mitochondria and whole tissues should be homogenized in PBS, while cultured cell pellets should be suspended in PBS. The protein concentration should then be determined using a standard method such as BCA method. Then, use PBS to adjust the sample concentrations as follows:

5.3 mg/mL for mitochondria

23.7 mg/mL for tissue homogenates

15 mg/mL for cultured cells

(Approximate numbers of cells/mg protein are given in the Frequently Asked Questions section).

10.2 Solubilize intact, functional PDH by adding Detergent to the samples as described below.

Component	Purified mitochondria at 5.3 mg/mL	Tissue homogenates at 23.7 mg/mL	Cultured cells at 15 mg/mL
Sample	19 volumes	19 volumes	9 volumes
Detergent	1 volume	1 volume	1 volume
Final Protein Concentration (mg/mL)	5.0	22.5	13.5

10.3 Incubate on ice for 10 minutes.

10.4 Centrifuge in a tabletop centrifuge for 10 minutes at 4°C as specified below. Carefully collect and save the supernatant. Discard the pellet.

Sample Type	RFC (x g)
Purified mitochondria	5,000
Tissue homogenates	1,000
Cultured cells	1,000

10.5 Dilute all samples to the desired concentration in Incubation Solution. Table 1 below shows the working range for the assay using various samples. The working range for your sample set should be confirmed by testing a representative reference control sample at a series of dilutions across the expected working range. Results from individual experimental samples can then be compared directly when tested at concentrations within the working range.

Sample Type	Recommended amount
Tissue extracts	0.5 - 25 µg / 200 µL
Cultured cell extracts†	0.5 - 50 µg / 200 µL

Table 1. Typical ranges of measurement.

† Mitochondrial PDH quantity is controlled by cellular metabolism. Consequently, cells with different metabolic requirements, such as those derived from different tissues, vary widely in their PDH amount. Additionally, cells of the same kind but cultured in different growth conditions show similar effects. For example, cells grown in glucose-rich media derive most of their energy by glycolysis. Cells grown in carbon sources which promote oxidative phosphorylation (such as galactose/glutamine), upregulate mitochondrial enzymes, including PDH. Ultimately, the cell type and growth conditions must be chosen carefully to obtain PDH quantity measurements.

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

12.1 Load wells at 200 µL per well with samples prepared in Section 10. Include a control (normal) sample as a positive control. Also include a buffer control (200 µL Incubation Solution without sample) as a null or background reference.

12.2 Cover/seal the plate and incubate for 3 hours at room temperature.

12.3 Wash the plate as follows: Empty the wells by turning the plate over a receptacle and firmly shaking out the well contents in one rapid downward motion. Rapidly add 300 µL 1X Stabilizer to each well. The wells must not become dry during any step. Repeat this wash once more for a total of two washes in 1X Stabilizer. After the last wash strike the microplate surface onto paper towels to remove excess liquid.

12.4 Add 200 µL of 1X Detector Antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature.

12.5 Repeat the wash procedure in step 12.3 except this time use 1X Buffer (without Stabilizer) for a total of two washes in 1X Buffer.

12.6 Add 200 µL of 1X HRP Label to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. Meanwhile prepare the microplate spectrophotometer using the parameters described below.

12.7 Repeat the wash procedure in step 12.5, but perform a total of three washes with 1X Buffer.

12.8 Rapidly add 200 µL HRP Development solution to each empty well and record (at room temperature) blue colour development in the prepared microplate reader immediately

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 15 min
Interval:	20 sec to 1 min
Shaking:	Shake between readings

Alternative– At a user defined color development time, record the endpoint OD data at (i) 600 nm or (ii) stop the reaction by adding 50 µL stop solution (1 N HCl) to each well and record OD at 450 nm.

13. TYPICAL DATA

Examine the colour development over time in each well. Under the conditions stated above the colour development should be linear over the 30 minute time period of measurement. Subtract the initial absorbance reading from the final absorbance reading to determine the amount of PDH in each well. This amount should always be related to a control or normal sample to obtain the relative amount of PDH in experimental samples.

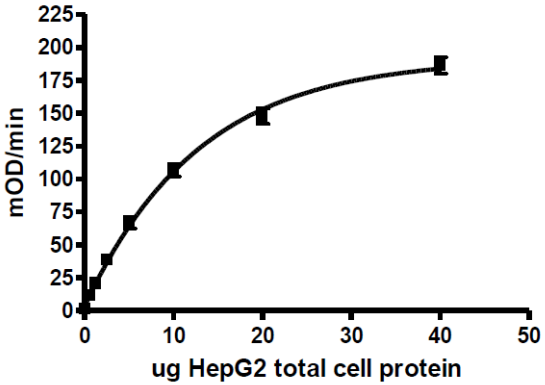


Figure 1 is an example of the quantity of PDH capture from a HepG2 cultured cell lysate. The sample was diluted to show that over this range of concentrations that can be used. Each sample was measured in 6 replicates. Bars show standard deviations.

REPRODUCIBILITY

Typical intra-assay variation (same day, same sample) <15%

14. SPECIES REACTIVITY

This assay has been developed for use with Human samples but bovine, mouse, and rat materials are also compatible. Other species have not been tested.

15. FREQUENTLY ASKED QUESTIONS

How do I grow and prepare cultured cell samples?

The amount of PDH in cells from different origins differs greatly. Cells grown in glucose have a lower activity than those grown in galactose/glutamine. Consequently, cell type and growth conditions are a large factor in PDH activity measured.

Approximately how much protein is yielded from my plate of cells?

We find the following typical yield of cells from a single confluent 177 cm^2 plate:

Human fibroblasts	1 x 10^7 cells	1.5 mg total protein
Human HepG2	2 x 10^7 cells	3 mg total protein

It is recommended that you accurately determine from your first confluent plate the number of cells and the total protein yield.

16. NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)