ab1814145 Human PDH E1 alpha protein SimpleStep ELISA® Kit

For the quantitative measurement of PDH E1 alpha protein in human cell and tissue extracts.

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

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1. Overview

PDH E1 alpha protein *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of PDH E1 alpha protein protein in human cell and tissue extracts.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

The pyruvate dehydrogenase complex performs the decarboxylation of pyruvate into acetyl CoA, a critical function in metabolism, linking glycolysis and oxidative phosphorylation in mitochondria. The enzyme is composed of multiple copies of three enzymes: pyruvate dehydrogenase (E1), dihydrolipoamide transacetylase (E2) and dihydrolipoamide dehydrogenase (E3). The E1 enzyme is a tetramer of two alpha (PDHA1) and two beta (PDHB) subunits and is present in 30 copies in the PDH complex.

The activity of PDH is regulated by reversible phosphorylation of three serine residues on the PDHA1 subunit at phospho S232, phospho S293, and phospho S300. ELISA assays to measure total PDHA1, phospho 232, phospho S293, and phospho S300 are available from Abcam (ab115342, ab115343, ab115344, ab115345). The phosphorylation of these sites is catalyzed by PDH kinases (PDK). There are four known PDK isoforms, distributed differently in tissues. Their expressions are regulated differently by factors such as

starvation, hypoxia and utilization of glucose and fatty acids in various tissues. Dephosporylation, to restore the activity of PDH, is catalyzed by PDH phosphatases (PDP). There are two known isoforms of PDP; PDP1 is present in high levels in skeletal muscle and PDP2 in liver and adipocytes. Functional PDH kinases and phosphatases are available from Abcam (ab110359, ab110354, ab110355, ab110356).

5 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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

⇩

Add 50 µL standard or sample to appropriate wells

⇩

Add 50 µL Antibody Cocktail to all wells

⇩

Incubate at room temperature for 1 hour

⇩

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

⇩

Add 100 µL TMB Development Solution to each well and incubate for 20 minutes.

⇩

Add 100 µL Stop Solution and read OD at 450 nm

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.

– We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.

– Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.

– Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.

– All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. 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.

6. Materials Supplied

Item	Quantity	Storage Condition
Human PDH E1 alpha protein Capture Antibody 10X	600 µL	+4°C
Human PDH E1 alpha protein Detector Antibody 10X	600 µL	+4°C
Human PDH E1 alpha protein Lyophilized Recombinant Protein	2 Vials	+4°C
4X Antibody Diluent EB	6 mL	+4°C
10X Wash Buffer LM	20 mL	+4°C
2X Cell Extraction Buffer LM	10 mL	+4°C
Sample Diluent NS*	12 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

*Sample Diluent NS is provided but not necessary for this product.

7. Materials Required, Not Supplied

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

– Microplate reader capable of measuring absorbance at 450 or 600 nm.
– Method for determining protein concentration (BCA assay recommended).
– Deionized water.
– Multi- and single-channel pipettes.
– Tubes for standard dilution.
– Plate shaker for all incubation steps.
– Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. Technical Hints

– Samples generating values higher than the highest standard 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, standard 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 11).
– All samples should be mixed thoroughly and gently.
– Avoid multiple freeze/thaw of samples.
– Incubate ELISA plates on a plate shaker during all incubation steps.
– When generating positive control samples, it is advisable to change pipette tips after each step.

- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- 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, control or standard 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. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

–   Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1   1X Cell Extraction Buffer LM:

Prepare 2X Cell Extraction Buffer LM by diluting 2X Cell Extraction Buffer LM to 1X with deionized water. To make 10 mL 2X Cell Extraction Buffer LM combine 5 mL deionized water, 5 mL 2X Cell Extraction Buffer LM. Mix thoroughly and gently. If required protease inhibitors can be added.

9.2   1X Wash Buffer LM:

Prepare 1X Wash Buffer LM by diluting 10X Wash Buffer LM with deionized water. To make 50 mL 1X Wash Buffer LM combine 5 mL 10X Wash Buffer LM with 45 mL deionized water. Mix thoroughly and gently.

9.3   Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in 4X Antibody Diluent EB and 1X Wash Buffer LM. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 1.65 mL 1X Wash Buffer LM and 750 µL of 4X Antibody Diluent EB. Mix thoroughly and gently.
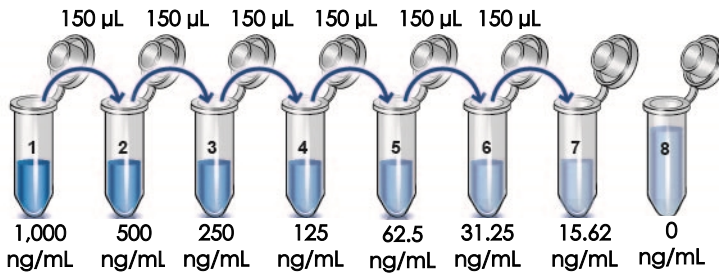
10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1.1 IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the PDH E1 standard by adding that volume of 1X Cell Extraction Buffer PTR indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the PDH E1 standard by adding 350 µL 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes and mix gently. This is the 1,000 ng/mL Stock Standard Solution.

10.1.2 Label eight tubes, Standards 1– 8.

10.1.3 Add 150 µL 1X Cell Extraction Buffer LM into tube number 1 and 150 µL of 1X Cell Extraction Buffer LM into numbers 2-8.

10.1.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

| 150 µL | 150 µL | 150 µL | 150 µL | 150 µL | 150 µL | | |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |

| 1,000 ng/mL | 500 ng/mL | 250 ng/mL | 125 ng/mL | 62.5 ng/mL | 31.25 ng/mL | 15.62 ng/mL | 0 ng/mL |

11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Hela cell extract	8-125 µg/mL
HepG2 Cell Lysate	8-125 µg/mL
Human Liver Homogenate (HLH) Lysate	16-500 µg/mL
MCF7 Cell Lysate	16-500 µg/mL

11.1 Preparation of extracts from cell pellets:

11.1.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.1.2 Rinse cells twice with PBS.

11.1.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer LM.

11.1.4 Incubate on ice for 20 minutes.

11.1.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.1.6 Transfer the supernatants into clean tubes and discard the pellets.

11.1.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.1.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer LM.

11.2 Preparation of extracts from adherent cells by direct lysis (alternative protocol):

11.2.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.2.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer LM directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer LM per confluent 15 cm diameter plate).

11.2.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.2.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.2.5 Transfer the supernatants into clean tubes and discard the pellets.

11.2.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.2.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer LM.

11.3 Preparation of extracts from tissue homogenates:

11.3.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.3.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer LM. For lower amounts of tissue adjust volumes accordingly.

11.3.3 Incubate on ice for 20 minutes.

11.3.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.3.5 Transfer the supernatants into clean tubes and discard the pellets.

11.3.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer LM.

12. 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 plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two 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).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature prior to use.

– We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer LM into each well. Wash Buffer LM should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 20 minutes in the dark on a plate shaker set to 400 rpm.
Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. <u>Note</u>: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed

time in the microplate reader prepared with the following settings:

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

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

**13.10** Analyze the data as described below.

14. Calculations

14.1   Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2   **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

Δ   **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3   Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4   Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.
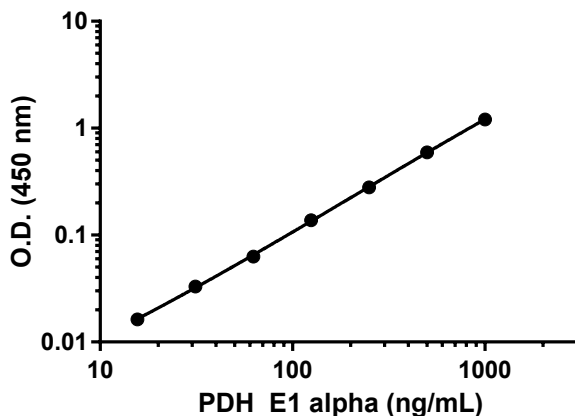
Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.05	0.05	0.05
15.62	0.07	0.07	0.07
31.25	0.09	0.08	0.09
62.5	0.12	0.11	0.12
125	0.2	0.19	0.2
250	0.33	0.33	0.33
500	0.65	0.65	0.65
1,000	1.24	1.29	1.27

Figure 1. Example of human PDH E1 alpha protein standard curve in 1X Cell extraction buffer LM. The PDH E1 alpha protein standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 12 ng/mL. The MDD was determined by calculating the mean of zero standard replicates (n=37) and adding 2 standard deviations then extrapolating the corresponding concentration.

RECOVERY –

Three concentrations of PDH E1 alpha protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
50% Cell Culture Media	135	137 – 134
10% Fetal Bovine Serum	135	130 – 129
10% Human Serum	144	152 - 129

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.
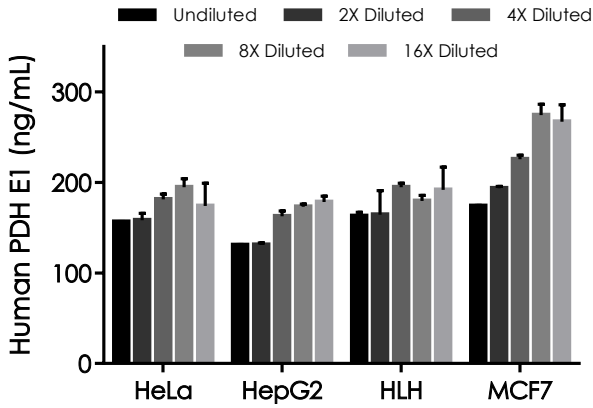
Native PDH E1 alpha protein was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 1X Cell Extraction Buffer LM.

Dilution Factor	Interpolated value	125 µg/mL HeLa Lysate	125 µg/mL HepG2 Lysate	500 µg/mL HLH Lysate	500 µg/mL MCF7 Lysate
Undiluted	ng/mL	156	131	163	173
	% Expected value	100	100	100	100
2	ng/mL	79	66	82	97
	% Expected value	101	100	101	111
4	ng/mL	45	41	49	56
	% Expected value	116	124	120	130
8	ng/mL	24	22	23	34
	% Expected value	125	132	110	158
16	ng/mL	11	11	12	17
	% Expected value	111	136	118	154

PRECISION –

Mean coefficient of variations of interpolated values of PDH E1 alpha protein from three concentrations of HeLa extracts within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	2	5
CV(%)	7.7	3.8

Figure 2. Interpolated concentrations of native PDH E1 alpha protein in human Hela, HepG2, human liver homogenate (HLH) and MCF7 cell extract. The concentrations of PDH E1 alpha protein were measured in duplicate and interpolated from the PDH E1 alpha protein standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

17. Assay Specificity

This kit recognizes both native and recombinant human PDH E1 alpha protein cell and tissue extract samples only.

Serum, plasma, urine, saliva, and cell culture supernatant samples have not been tested with this kit.

18. Species Reactivity

This kit recognizes human PDH E1 alpha protein protein.

Other species reactivity not determined.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer LM (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

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)