



Pyruvate Dehydrogenase Microplate Assay Kit User Manual

Catalog # FTA0070

(Version 1.2D)

Detection and Quantification of Pyruvate Dehydrogenase (PDH)

Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
IV. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

I. INTRODUCTION

Pyruvate dehydrogenase (PDH) is a mitochondrial enzyme that catalyzes the conversion of pyruvate to acetyl-CoA and CO₂, and also links the tricarboxylic acid (TCA) and glycolysis pathways. The enzyme is inhibited by phosphorylation and activated by dephosphorylation. Mutations in PDH have been linked to pyruvate dehydrogenase deficiency (causing lactic acidosis and neurologic dysfunctions) and Leigh syndrome. PDH has also been implicated in oncogene-induced senescence. PDH measurements can provide insights into metabolic functions and oncogenesis. Pyruvate Dehydrogenase Activity Microplate Assay Kit provides a simple and direct procedure for measuring pyruvate dehydrogenase activity levels in a variety of samples. The assay is initiated with the enzymatic hydrolysis of pyruvic acid by PDH. The enzyme catalysed reaction products can be measured at a colorimetric readout at 570 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	1.2 ml x 1	4 °C
Assay Buffer III	20 ml x 1	4 °C
Substrate Dilution	10 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Substrate: add 9 ml Substrate Dilution to dissolve before use.

Dye Reagent: add 10 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For serum or plasma samples

Add 0.9 ml Assay Buffer I for 0.1 ml serum or plasma; mix; centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer I on ice, centrifuged at 10000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For mitochondria

Weigh out 0.1 g tissue, homogenize with 0.99 ml Assay Buffer I and 10 µl Assay Buffer II on ice, transfer the solution into a new centrifuge tube, 10000g 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times). Then add 800 µl distilled water, mix, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Blank	Standard
Sample	10 µl	--	--
Substrate	90 µl	--	--
Standard	--	--	100 µl
Distilled water	--	100 µl	--
Dye Reagent	100 µl	100 µl	100 µl
Mix, incubate at 37 °C for 2 minutes, measured at 570 nm and record the absorbance.			

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VI. CALCULATION

Unit Definition: One unit of PDH activity is defined as the enzyme produce 1 nmol NADH per minute.

1. According to the protein concentration of sample

$$\begin{aligned}\text{PDH (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / OD_{\text{Standard}} / (V_{\text{Sample}} \times C_{\text{Protein}}) \\ &\quad / T \\ &= 2000 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / OD_{\text{Standard}} / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{PDH (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / OD_{\text{Standard}} / (V_{\text{Sample}} \times W / \\ &\quad V_{\text{Assay}}) / T \\ &= 2000 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / OD_{\text{Standard}} / W\end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned}\text{PDH (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / OD_{\text{Standard}} / (V_{\text{Sample}} \times N / \\ &\quad V_{\text{Assay}}) / T \\ &= 2000 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / OD_{\text{Standard}} / N\end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned}\text{PDH (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / OD_{\text{Standard}} / V_{\text{Sample}} / T \\ &= 2000 \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / OD_{\text{Standard}}\end{aligned}$$

C_{Standard} : the standard concentration, 400 $\mu\text{mol/L}$ = 400 nmol/ml;

V_{Standard} : the volume of standard, 100 μl = 0.1 ml;

C_{Protein} : the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

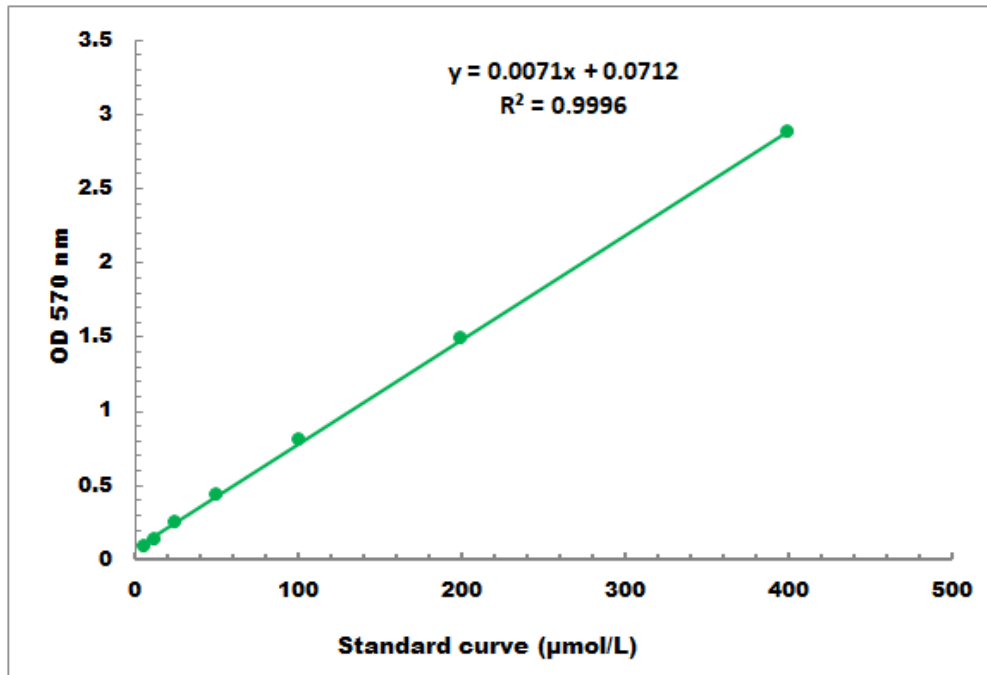
V_{Sample} : the volume of sample, 0.01 ml;

V_{Assay} : the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 4 µmol/L - 400 µmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES