（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K500-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader（330-350 nm）

Detection range：0.67-27.73 U/L

Elabscience® Pyruvate Decarboxylase（PDC）Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary 3

Intended use 4

Detection principle 4

Kit components & storage 4

Materials prepared by users 5

Reagent preparation 5

Sample preparation 6

The key points of the assay 7

Operating steps 7

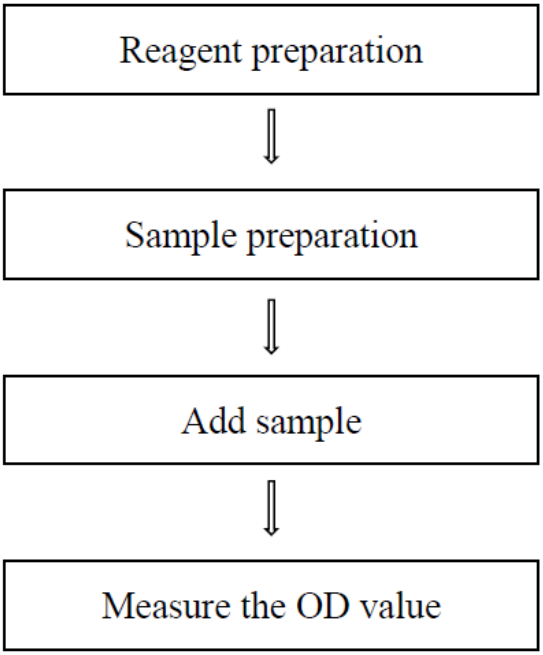
Calculation 8

Appendix I Performance Characteristics 9

Appendix Π Example Analysis 10

Statement 11

Assay summary



Intended use

This kit can be used to measure pyruvate decarboxylase (PDC) activity in serum, plasma, tissue and cell samples.

Detection principle

Pyruvate decarboxylase (PDC) mainly exists in yeast and is one of the key enzymes in ethanol fermentation. PDC catalyzes pyruvate decarboxylate to acetaldehyde, which reacts under the action of ethanol dehydrogenase (ADH), and catalyzes NADH to convert into NAD^+. NADH has a characteristic absorption peak at 340 nm. The PDC activity can be calculated by measuring the OD value at 340 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL ×1 vial	20 mL ×1 vial	-20 ℃, 12 months shading light
Reagent 2	Substrate A	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months shading light
Reagent 3	Enzyme Reagent	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months shading light
Reagent 4	Substrate B	Powder ×1 vial	Powder ×2 vials	-20 ℃, 12 months shading light
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (330-350 nm, optimum wavelength: 340 nm)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate A working solution：
Dissolve a vial of substrate A with 5 mL double distilled water. Keep it on the ice protected from light for use. Store at -20 ℃ for 3 days protected from light.

③ The preparation of enzyme working solution：
Dissolve a vial of enzyme reagent with 1.2 mL double distilled water. Keep it on the ice protected from light for use. Store at -20 ℃ for 7 days protected from light.

④ The preparation of substrate B working solution：
Dissolve a vial of substrate B with 1.2 mL double distilled water. Keep it on the ice protected from light for use. Store at -20 ℃ for 3 days protected from light.

⑤ The preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 1500 μL of reaction working solution (mix well 500 μL of substrate A working solution and 500 μL of enzyme working solution and 500 μL of substrate B working solution). The reaction working solution should be prepared on spot.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 ℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4 ℃.

④ Centrifuge at 12000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4 ℃.

④ Centrifuge at 12000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	25-50
10% Mouse kidney tissue homogenate	25-50
10% Mouse heart tissue homogenate	25-50
Rat serum	1
Rabbit plasma	1
HL-60 cell	3-5

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① All reagents are stored strictly with shading light and avoid from repeated freezing and thawing.

② The change of OD per unit time (min) should be controlled within 0.2.

Operating steps

① Blank well: Add 20 μL of double distilled water to the wells.
Sample well: Add 20 μL of sample to the wells.

② Add 120 μL of buffer solution into each well.

③ Add 60 μL of reaction working solution into each well.

④ Mix fully, measure the OD value of each well at 1 min and 3 min respectively at 340 nm with microplate reader, recorded as A_1, A_2, $\triangle A = A_1 - A_2$.

Calculation

The sample:

1. Serum (plasma) sample:

Definition: The amount of PDC in 1 L liquid sample per 1 minute that hydrolyze the NADH to produce 1 μmol NAD at 37 °C is defined as 1 unit.

$$\text{PDC activity (U/L)} = (\Delta A_{sample} - \Delta A_{blank}) \div (\varepsilon \times d) \div T \times f \times 10^6$$

2. Tissue and cell sample:

Definition: The amount of PDC in 1 g tissue or cell protein per 1 minute that hydrolyze the NADH to produce 1 μmol NAD at 37 °C is defined as 1 unit.

$$\text{PDC activity (U/gprot)} = (\Delta A_{sample} - \Delta A_{blank}) \div (\varepsilon \times d) \div C_{pr} \div T \times f \times 10^6$$

[Note]

ΔA_{sample}: The change OD value of sample well, A_1-A_2.

ΔA_{blank}: The change OD value of blank well, A_1-A_2.

ε: Molar extinction coefficient of NADH, 6.22×10^3 L/(mol•cm).

d: The optical path of microplate, 0.6 cm.

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

T: The reaction time, 2 min.

10^6: 1 mol = 10^6 μmol.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.70	13.50	22.60
%CV	3.2	2.7	2.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.70	13.50	22.60
%CV	2.8	3.1	3.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	7.4	19.3	25.5
Observed Conc. (U/L)	7.8	20.7	26.0
Recovery rate (%)	106	107	102

Sensitivity

The analytical sensitivity of the assay is 0.67 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix Π Example Analysis

Example analysis:

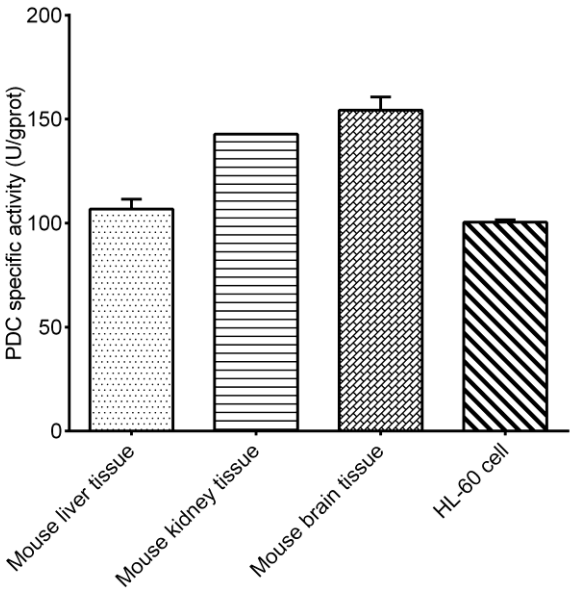
For 10% mouse liver tissue homogenate, dilute for 50 times, and carry the assay according to the operation steps. The results are as follows:

The A_1 of the blank well is 1.054, the A_2 of the blank well is 1.053, the A_1 of the sample well is 0.674, the A_2 of the sample well is 0.448, the concentration of protein in sample is 14.18 gprot/L, and the calculation result is:

PDC activity (U/gprot) = ((0.674-0.448)-(1.054-1.053)) ÷ (6220 × 0.6)÷ 14.18 ÷ 2 × 50 × 10^6

= 106.29 U/gprot

Detect 10% mouse liver tissue homogenate (the concentration of protein is 14.18 gprot/L, dilute for 50 times), 10% mouse kidney tissue homogenate (the concentration of protein is 13.37 gprot/L, dilute for 50 times), 10% mouse brain tissue homogenate (the concentration of protein is 5.99 gprot/L, dilute for 50 times), and HL-60 cell (the concentration of protein is 5.99 gprot/L, dilute for 3 times) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.