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

**Catalog No：E-BC-K650-M**

**Specification：96T(40 samples)**

**Measuring instrument：Microplate reader（440-460 nm）**

**Detection range：0.28-168.91 U/L**

# **Elabscience®** Pyruvate Dehydrogenase (PDH) 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.

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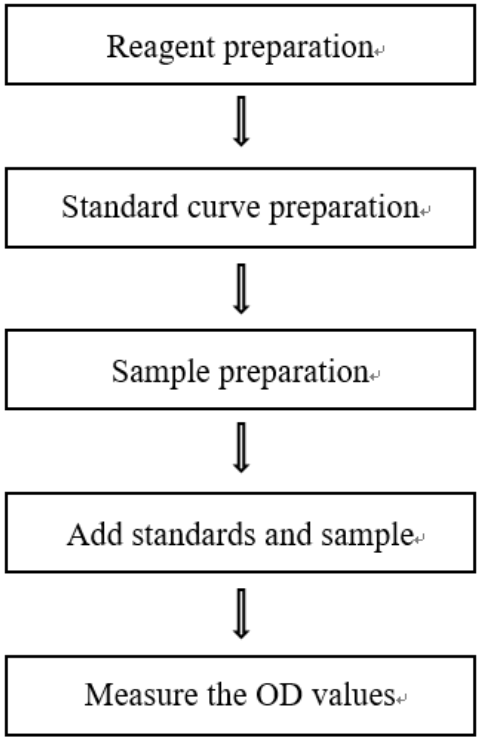
**Assay summary**



## Intended use

This kit can measure pyruvate dehydrogenase (PDH) activity in serum, plasma, urine, animal tissue and cell samples.

## Detection principle

Pyruvate dehydrogenase (PDH) catalyzed the oxidative decarylation of pyruvate into acetyl CoA, which plays an important role in the metabolism of mitochondrial respiratory chain and is associated with the aging of cancer genes. Pyruvate can be decarboxylated by PDH under the action of CoA. Meanwhile, $NAD^+$ is reduced to NADH, Which under the action of hydrogen transmitter, transfer electrons to WST-8 to produce the yellow product. The activity of PDH can be calculated by measuring the change of absorbance value at 450 nm.

## Kit components & storage

| Item | Component | Size (96 T) | Storage |
|---|---|---|---|
| Reagent 1 | Extraction Solution | 50 mL ×2 vials | -20℃, 12 months |
| Reagent 2 | Buffer Solution | 40 mL ×1 vial | -20 ℃, 12 months, shading light |
| Reagent 3 | Substrate A | Powder ×2 vials | -20 ℃, 12 months, shading light |
| Reagent 4 | Substrate B | Powder ×2 vials | -20 ℃, 12 months, shading light |
| Reagent 5 | Chromogenic Agent | 6 mL ×1 vial | -20 ℃, 12 months, shading light |
| Reagent 6 | Standard | Powder ×2 vials | -20 ℃, 12 months, shading light |
| | 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:**

Centrifuge, Microplate reader (440-450 nm, optimum wavelength: 450 nm)

**Reagents:**

Double distilled water

## Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of substrate A working solution:
Dissolve one vial of substrate A with 500 μL of double distilled water, mix well to dissolve. Keep substrate A working solution on ice protected from light during use. Storage at -20℃ for 3 days protected from light, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of buffer working solution:
Before testing, please prepare sufficient buffer working solution according to the test wells. For example, prepare 505 μL of buffer working solution (mix well 500 μL of buffer solution and 5 μL of substrate A working solution). The buffer working solution should be used up in the same day.

④ The preparation of substrate B working solution:
Dissolve one vial of substrate B with 1.6 mL of double distilled water, mix well to dissolve. Keep substrate B working solution on ice during use. Storage at -20℃ for 7 days protected from light.

⑤ The preparation of 0.5 mmol/L standard solution:
Dissolve one vial of standard with 1 mL of double distilled water, mix well to dissolve. Keep 0.5 mmol/L standard solution on ice during use. Storage at -20℃ for 7 days protected from light.

⑥ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions

after use.

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.05, 0.1, 0.2, 0.25, 0.3, 0.4, 0.5 mmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (mmol/L)** | **0** | **0.05** | **0.1** | **0.2** | **0.25** | **0.3** | **0.4** | **0.5** |
| **0.5 mmol/L standard (μL)** | 0 | 20 | 40 | 80 | 100 | 120 | 160 | 200 |
| **Double distilled water (μL)** | 200 | 180 | 160 | 120 | 100 | 80 | 40 | 0 |

## Sample preparation

### ① Sample preparation

**Serum and plasma:** detect directly (if the sample is turbidity, centrifugation at 12000 g for 10 min before use). 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 PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4℃.

④ Centrifuge at 12000×g for 10 min at 4℃ 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).

**Cell (adherent or suspension) samples:**

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

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 12000×g for 10 min at 4℃ 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% Rat liver tissue homogenate | 2-3 |
| 10% Rat kidney tissue homogenate | 1 |
| 10% Rat heart tissue homogenate | 1 |
| 10% Rat brain tissue homogenate | 1 |
| 10% Rat lung tissue homogenate | 1 |
| 10% Mouse liver tissue homogenate | 2-3 |
| 10% Mouse kidney tissue homogenate | 1 |
| 10% Mouse lung tissue homogenate | 1 |
| 1×10^6 HL-60 cells | 1 |
| Rat serum | 1 |
| Rat plasma | 1 |
| Human urine | 1 |

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

## Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations to the corresponding wells.

Control well: Add 20 μL of sample to the corresponding wells.

Sample well: Add 20 μL of sample to the corresponding wells.

② Add 140 μL of buffer working solution to each well.

③ Add 20 μL of substrate B working solution to standard well and sample well. Add 20 μL of double distilled water to control well

④ Add 20 μL of chromogenic agent to each well.

⑤ Measure the OD value of each well at 20 s and 3 min 20 s respectively at 450 nm with microplate reader, recorded as $A_1$, $A_2$, $\triangle A = A_1 - A_2$.Plot the standard curve with $A_2$ values.

## Calculation

**The standard curve**:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

**The sample**:

**1. Serum (plasma) sample:**

**Definition:** The amount of PDH in 1 L liquid sample per 1 minute that hydrolyze the substrate to produce 1 μmol product at 37°C is defined as 1 unit.

$$\text{PDH activity (U/L)} = (\Delta A_{sample} - \Delta A_{control} - b) \div a \div T \times f \times 1000$$

**2. Tissue and cells samples:**

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

$$\text{PDH activity (U/gprot)} = (\Delta A_{sample} - \Delta A_{control} - b) \div a \div C_{pr} \div T \times f \times 1000$$

**[Note]**

$\Delta A_{sample}$: The change OD value of sample well, $A_2$-$A_1$.

$\Delta A_{control}$: The change OD value of control well, $A_2$-$A_1$.

T: The time of reaction, 3 min.

$C_{pr}$: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

1000: 1 mmol = 1000 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)** | 1.50 | 35.50 | 85.60 |
| **%CV** | 2.4 | 2.2 | 1.4 |

### Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| **Mean (U/L)** | 1.50 | 35.50 | 85.60 |
| **%CV** | 9.6 | 10.2 | 10.2 |

### 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 95%.

| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| **Expected Conc. (mmol/L)** | 0.08 | 0.23 | 0.37 |
| **Observed Conc. (mmol/L)** | 0.1 | 0.2 | 0.4 |
| **Recovery rate (%)** | 94 | 96 | 95 |

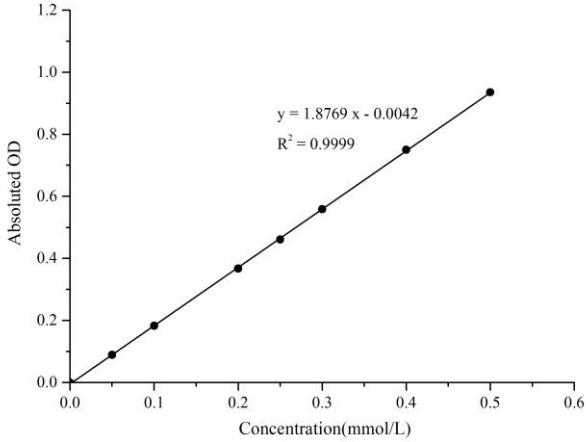
### Sensitivity

The analytical sensitivity of the assay is 0.28 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.

**2. Standard curve:**

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

| **Concentration (mmol/L)** | **0** | **0.05** | **0.1** | **0.2** | **0.25** | **0.3** | **0.4** | **0.5** |
|---|---|---|---|---|---|---|---|---|
| **Average OD** | 0.058 | 0.147 | 0.241 | 0.425 | 0.519 | 0.616 | 0.808 | 0.993 |
| **Absoluted OD** | 0.000 | 0.090 | 0.184 | 0.367 | 0.461 | 0.559 | 0.750 | 0.936 |



# Appendix II Example Analysis

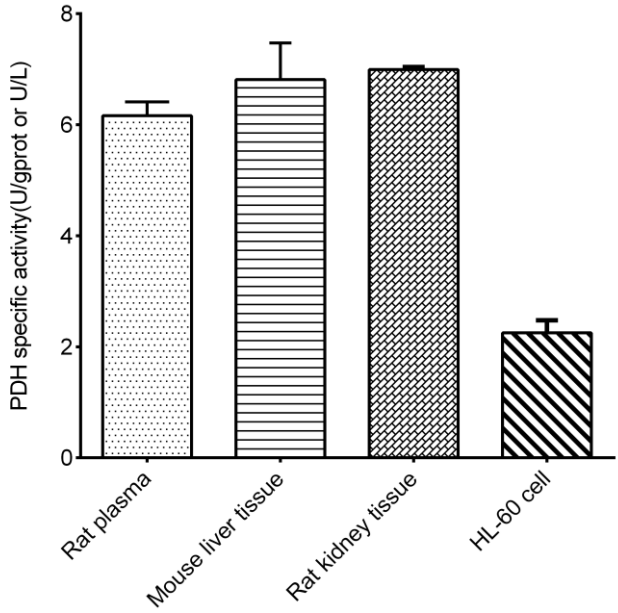
**Example analysis:**

For 10% rat kidney tissue homogenate, carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 1.8769 x - 0.0042, the $A_1$ of the control well is 0.175, the $A_2$ of the control well is 0.177, $\Delta A_{Control}$ = 0.177–0.175 = 0.002. The $A_1$ of the sample well is 0.217. the $A_2$ of the sample well is 0.602, $\Delta A_{Sample}$ = 0.602–0.217 = 0.385, the concentration of protein in sample is 10.08 gprot/L, and the calculation result is:

PDH activity (U/gprot) = $(0.385 - 0.002 + 0.0042) \div 1.8769 \div 10.08 \div 3 \times 1000 = 6.822$ U/gprot

Detect 10% rat kidney tissue homogenate (the concentration of protein is 10.08 gprot/L), 10% mouse liver tissue homogenate (the concentration of protein is 14.91 gprot/L), HL-60 cell (the concentration of protein is 1.12 gprot/L) and rat plasma 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.