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

Catalog No：E-BC-K130-S

Specification：50 Assays (48 samples)/ 100 Assays (96 samples)

Measuring instrument：Spectrophotometer（480-520 nm）

Detection range：0.006-2.0 μmol/mL

Elabscience® Pyruvic Acid Colorimetric 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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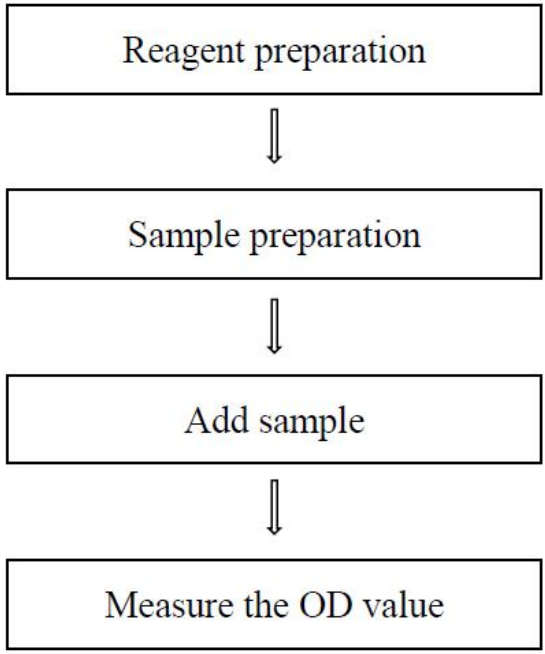
Assay summary

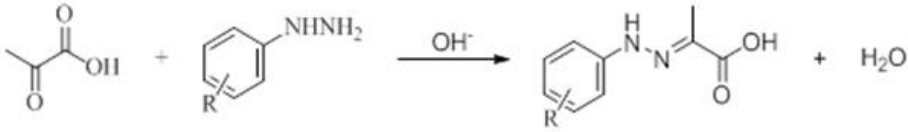


Intended use

This kit can be used to measure pyruvic acid content in serum, plasma and tissue samples.

Detection principle

Pyruvic acid can react with chromogenic agent and the reaction product is reddish brown in alkaline solution. The depth of color is directly proportional to the pyruvate content. The pyruvate content can be calculated by measuring the OD value at 505 nm.



Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Clarificant	12 mL × 1 vial	12 mL × 1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Alkali Reagent	50 mL × 3 vials	50 mL × 6 vials	2-8°C, 12 months
Reagent 4	2 μmol/mL Sodium Pyruvate Standard	1.6 mL × 1 vial	1.6 mL × 2 vials	2-8°C, 12 months

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 (480-520 nm, optimum wavelength: 505 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of 0.2 μmol/mL sodium pyruvate standard solution：
For each well, prepare 100 μL of 0.2 μmol/mL sodium pyruvate standard solution (mix well 10 μL of 2 μmol/mL sodium pyruvate standard and 90 μL of double distilled water). Store at 2-8°C for 7 days.

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°C 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 PBS(0.01 M，pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min 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, E-BC-K168-S, E-BC-K165-S).

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Mouse serum	1
Mouse plasma	1
10% Mouse liver tissue homogenization	1
10% Rat kidney tissue homogenization	1
10% Rat heart tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

1. For serum (plasma) sample

① Blank tube: Add 0.1 mL of double distilled water to 5 mL EP tube.
Standard tube: Add 0.1 mL of 0.2 μmol/mL sodium pyruvate standard solution to 5 mL EP tube.
Sample tube: Add 0.1 mL of sample to 5 mL EP tube.

② Add 0.5 mL of chromogenic agent to each tube and mix fully with a vortex mixer.

③ Incubate the tubes at 37°C for 10 min.

④ Add 2.5 mL of alkali reagent into each tube. Mix fully with vortex mixer for 5 s, then incubate the tubes at room temperature for 5 min.

⑤ Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 505 nm with 1 cm optical path cuvette.

2. For tissue sample

① Blank tube: Add 0.1 mL of double distilled water to 5 mL EP tube.
Standard tube: Add 0.1 mL of 0.2 μmol/mL sodium pyruvate standard solution to 5 mL EP tube.
Sample tube: Add 0.1 mL of sample to 5 mL EP tube.

② Add 0.1 mL of clarificant to each tube and mix fully with a vortex mixer.

③ Add 0.5 mL of chromogenic agent to each tube and mix fully with a vortex mixer.

④ Incubate the tubes at 37°C for 10 min.

⑤ Add 2.5 mL of alkali reagent into each tube. Mix fully with vortex mixer for 5 s, then incubate the tubes at room temperature for 5 min.

⑥ Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 505 nm with 1 cm optical path cuvette.

Calculation

The sample:

1. Serum (plasma) sample:

$$\text{Pyruvate content (μmol/mL)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

2. Tissue sample:

$$\text{Pyruvate content (μmol/mgprot)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f \div C_{pr}$$

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: Concentration of standard, 0.2 μmol/mL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (mgprot/mL).

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 (μmol/mL)	0.55	1.05	1.36
%CV	1.5	1.2	1.2

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 (μmol/mL)	0.55	1.05	1.36
%CV	1.4	1.6	1.5

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 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/mL)	0.7	1.3	1.7
Observed Conc. (μmol/mL)	0.7	1.3	1.8
Recovery rate (%)	99	98	103

Sensitivity

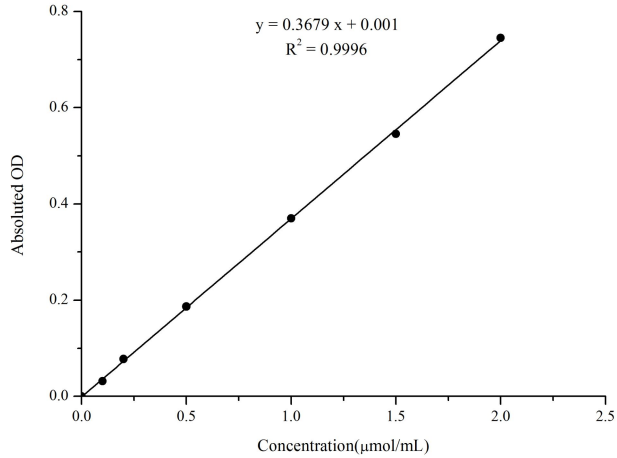
The analytical sensitivity of the assay is 0.006 μmol/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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 (μmol/mL)	0	0.1	0.2	0.5	1	1.5	2
Average OD	0.017	0.048	0.094	0.203	0.387	0.562	0.762
Absoluted OD	0	0.032	0.078	0.187	0.370	0.546	0.745



Appendix II Example Analysis

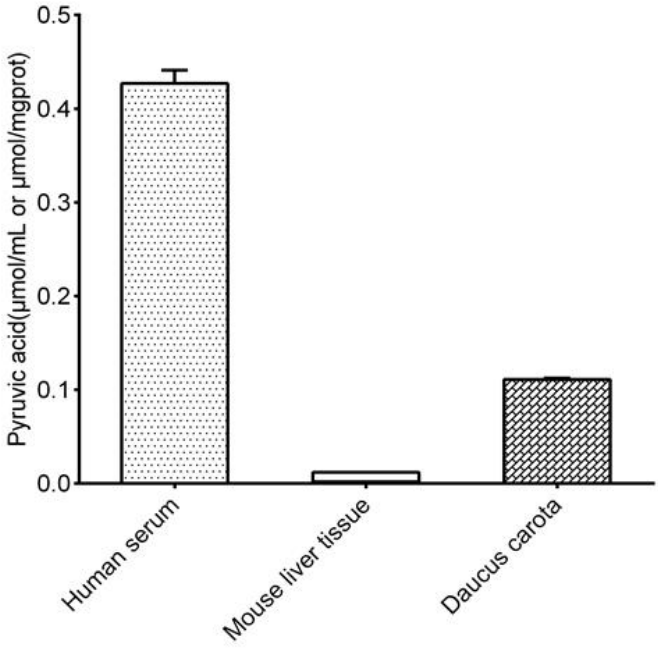
Example analysis：

Take 0.1 mL of human serum, carry the assay according to the operation steps. The results are as follows:

The average OD value of the sample is 0.174, the average OD value of the blank is 0.014, the average OD value of the standard is 0.089, and the calculation result is:

$$\text{Pyruvate content (μmol/mL)} = \frac{0.174 - 0.014}{0.089 - 0.014} \times 0.2 \times 1 = 0.43 \text{ μmol/mL}$$

Detect human serum, 10% mouse liver tissue homogenate (the concentration of protein in sample is 16.99 mgprot/mL), 10% daucus carota tissue homogenate (the concentration of protein in sample is 1.16 mgprot/mL) 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.