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

Catalog No：E-BC-K130-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（480-520 nm）

Detection range：0.003-2.0 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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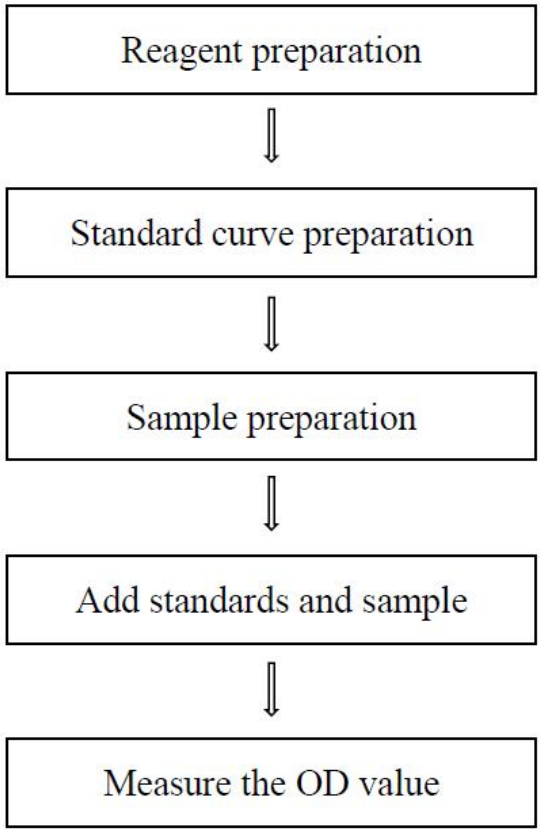
Assay summary

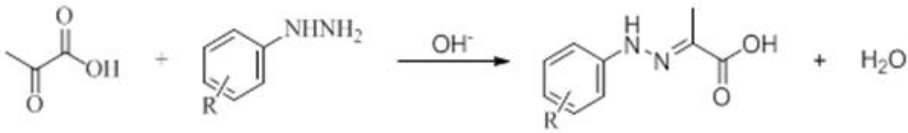


Intended use

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(48 T)	Size 2(96 T)	Storage
Reagent 1	Clarificant	0.6 mL × 1 vial	1.2 mL × 1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Alkali Reagent	10 mL × 1 vial	20 mL × 1 vial	2-8°C, 12 months
Reagent 4	2 μmol/mL Sodium Pyruvate Standard	1.6 mL × 1 vial	1.6 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	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 (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 standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 2 μmol/mL sodium pyruvate standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.6, 0.8, 1.2, 1.6, 2 μmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/mL)	**0**	**0.1**	**0.2**	**0.6**	**0.8**	**1.2**	**1.6**	**2.0**
2 μmol/mL standard (μL)	0	5	10	30	40	60	80	100
Double distilled water (μL)	100	95	90	70	60	40	20	0

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).

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 homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	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 Standard well: Add 15 μL of standard solution with different concentrations and 50 μL of chromogenic agent to the corresponding wells.

Sample well: Add 15 μL of sample and 50 μL of chromogenic agent to the corresponding wells.

② Mix fully with microplate reader for 10 s, then incubate at 37°C for 10 min.

③ Add 150 μL of alkali reagent into each well. Mix fully with microplate reader for 10 s, then incubate at room temperature for 5 min.

④ Measure the OD value of each well at 505 nm with microplate reader.

2. For tissue sample

① Standard well: Add 15 μL of standard solution with different concentrations to the corresponding wells.

Sample well: Add 15 μL of sample to the corresponding wells.

② Add 10 μL of clarificant and 50 μL of chromogenic agent to each well.

③ Mix fully with microplate reader for 10 s, then incubate at 37°C for 10 min.

④ Add 150 μL of alkali reagent into each well. Mix fully with microplate reader for 10 s, then incubate at room temperature for 5 min.

⑤ Measure the OD value of each well at 505 nm with microplate reader.

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:

$$\frac{\text{Pyruvic Acid}}{(\mu\text{mol/mL})} = (\Delta A_{505} - b) \div a \times f$$

2. Tissue sample:

$$\frac{\text{Pyruvic Acid}}{(\mu\text{mol/mgprot})} = (\Delta A_{505} - b) \div a \times f \div Cpr$$

[Note]

f: Dilution factor of sample before tested.

ΔA_{505}: $OD_{Sample} - OD_{Blank}$.

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	1.20	1.60
%CV	2.6	2.1	2.2

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	1.20	1.60
%CV	3.6	3.5	3.7

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. (μmol/mL)	0.15	0.7	1.4
Observed Conc. (μmol/mL)	0.1	0.7	1.3
Recovery rate (%)	97	96	92

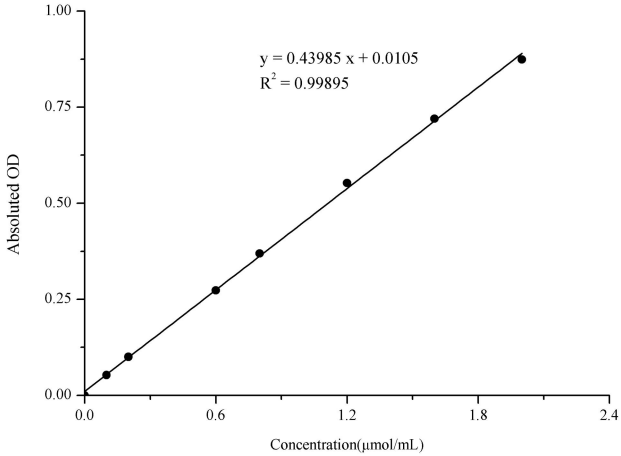
Sensitivity

The analytical sensitivity of the assay is 0.003 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:

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	0.1	0.2	0.6	0.8	1.2	1.6	2.0
OD value	0.047	0.105	0.145	0.326	0.419	0.601	0.768	0.896
	0.049	0.097	0.152	0.317	0.415	0.600	0.769	0.949
Average OD	0.048	0.101	0.148	0.321	0.417	0.601	0.768	0.922
Absoluted OD	0.000	0.053	0.100	0.273	0.369	0.553	0.720	0.874



Appendix II Example Analysis

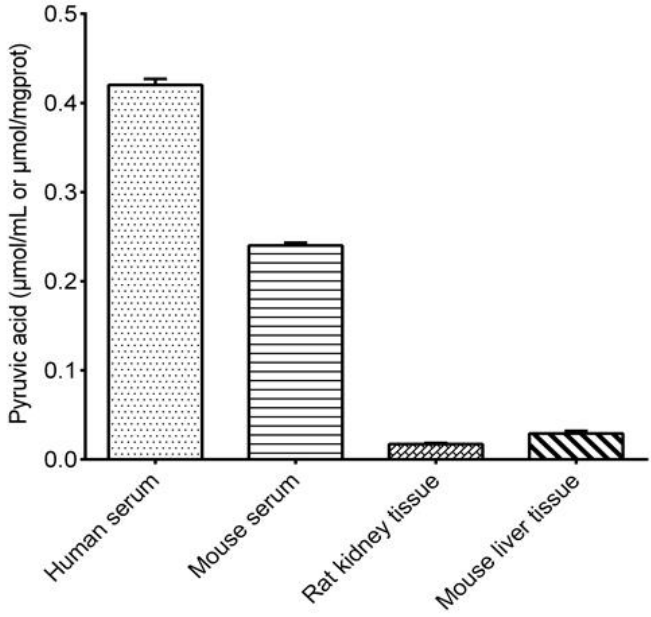
Example analysis：

Take 15 μL of human serum sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.4983 x + 0.0123, the average OD value of the sample is 0.269, the average OD value of the blank is 0.047, and the calculation result is:

$$\underset{(\mu mol/mL)}{\text{Pyruvic Acid}} = (0.269 - 0.047 - 0.0123) \div 0.4983 = 0.42\ \mu mol/mL$$

Detect human serum, mouse serum, 10% rat kidney tissue homogenate (the concentration of protein in sample is 10.36 mgprot/mL) and 10% mouse liver tissue homogenate (the concentration of protein in sample is 12.72 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.