Pyruvic Acid Colorimetric Assay Kit

Catalog No: E-BC-K130-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.006 μmol/mL

Detection range: 0.006-2.0 μmol/mL

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

General information

▲Intended use

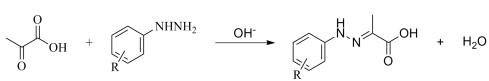
This kit can be used to measure pyruvate content of serum, plasma, tissue and cells samples.

▲Background

Pyruvic acid is the simplest of the α-keto acids, with a carboxylic acid and a ketone functional group. Pyruvic acid can be made from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis, or to fatty acids through a reaction with acetyl-CoA. It can also be used to construct the amino acid alanine and can be converted into ethanol or lactic acid via fermentation. Pyruvic acid supplies energy to cells through the citric acid cycle (also known as the Krebs cycle) when oxygen is present (aerobic respiration), and alternatively ferments to produce lactate when oxygen is lacking (lactic acid fermentation).

▲Detection principle

Pyruvic acid can react with chromogenic agent and the 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	Specification	Storage
Reagent 1	Clarificant	12 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Chromogenic Agent	60 mL ×1 vial	2-8℃ , 6 months, shading light
Reagent 3	Alkali Reagent	50 mL × 6 vials	2-8℃ , 6 months
Reagent 4	2 μmol/mL Sodium Pyruvate Standard	1.6 mL × 2 vials	2-8℃ , 6 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.			

▲Materials prepared by users

Instruments

Spectrophotometer (505 nm), Micropipettor, Vortex mixer, Incubator

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

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

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

Pre-assay preparation

▲Reagent preparation

Preparation of 0.2 μmol/mL sodium pyruvate standard solution:

Dillute reagent 4 with double distilled water for 10 times. Prepared the fresh solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.006-2.0 μmol/mL).

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).

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	505 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲For serum or plasma sample

Operating steps

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 reagent 2 to each tube and mix fully with a vortex mixer.
3. Incubate the tubes at 37℃ for 10 min.
4. Add 2.5 mL of reagent 3 into each tube. Mix fully with vortex mixer for 5 sec, 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.

Operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.1		
0.2 μmol/mL Sodium pyruvate standard solution (mL)		0.1	
Sample (mL)			0.1
Reagent 2 (mL)	0.5	0.5	0.5
Mix fully, incubate at 37℃ for 10 min			
Reagent 3 (mL)	2.5	2.5	2.5
Mix fully with vortex mixer for 5 sec, then incubate the tubes at room temperature for 5 min. 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.			

▲For tissue or cell sample

Operating steps

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 reagent 1 to each tube and mix fully with a vortex mixer.
3. Add 0.5 mL of reagent 2 to each tube and mix fully with a vortex mixer.
4. Incubate the tubes at 37℃ for 10 min.
5. Add 2.5 mL of reagent 3 into each tube. Mix fully with vortex mixer for 5 sec, 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.

Operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.1		
0.2 μmol/mL Sodium pyruvate standard solution (mL)		0.1	
Sample (mL)			0.1
Reagent 1 (mL)	0.1	0.1	0.1
Reagent 2 (mL)	0.5	0.5	0.5
Mix fully, incubate at 37 ℃ for 10 min			
Reagent 3 (mL)	2.5	2.5	2.5
Mix fully with vortex mixer for 5 sec, then incubate the tubes at room temperature for 5 min. 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

1. Serum (plasma) sample:

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

2. Tissue or cell sample:

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

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)

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.006-2.0 μmol/mL	Average intra-assay CV (%)	1.3
Sensitivity	0.006 μmol/mL	Average inter-assay CV (%)	1.5
Average recovery rate (%)	100		

▲Example analysis

Take 0.1 mL of human serum, carry the assay according to the operation table.

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:

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

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8℃ . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (2-8℃) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80℃ for a month.

▲Cells

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 3100 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4) or 0.9% NaCl.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.
Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.