Technical Manual

Pyruvic Acid Colorimetric Assay Kit

- Catalogue Code: MAES0105
- Size: 100 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.006-2.0 µmol/mL

Sensitivity:

0.006 µmol/mL

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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).

3. Intended Use

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

4. 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.

5. Kit Components & Storage

Item	Specification	Storage
Clarificant	12 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	60 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Alkaline Reagent	50 mL × 6 vials	2-8°C, 6 months
Sodium Pyruvate Standard (2 μmol/mL)	1.6 mL × 2 vials	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (510-520 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of 0.2 μ mol/mL sodium pyruvate standard solution: Dilute 2 μ mol/mL sodium pyruvate standard with double distilled water for 10 times. Prepared the fresh solution before use.

7. Sample Preparation:

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

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

3. Tissue:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (2-8°C) (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 and preserve on ice before detection.

4. Cell:
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 (3×10^6): normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) (μ L) =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 on ice for detection.

Sample Notes:
The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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);

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 505 nm

9. Operation Steps

The measurement of 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 μ mol/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 alkaline reagent into each tube. Mix fully with vortex mixer for 5s, 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.

The measurement of 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 μ mol/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 clarificatory 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 alkaline reagent into each tube. Mix fully with vortex mixer for 5s, 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 for Serum (Plasma)

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.1		
Sodium pyruvate standard solution (mL) (0.2 μ mol/mL)		0.1	
Sample (mL)			0.1
Chromogenic agent (mL)	0.5	0.5	0.5
Mix fully, incubate at 37°C for 10 min.			
Alkaline reagent (mL)	2.5	2.5	2.5
Mix fully with vortex mixer for 5s, 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.			

Operation Table for Tissue

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	0.1		
Sodium pyruvate standard solution (mL) (0.2 μ mol/mL)		0.1	
Sample (mL)			0.1
Clarificant (mL)	0.1	0.1	0.1
Chromogenic agent (mL)	0.5	0.5	0.5
Mix fully, incubate at 37°C for 10 min.			
Alkaline reagent (mL)	2.5	2.5	2.5
Mix fully with vortex mixer for 5s, 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.			

10. Calculations

1. Serum (plasma) sample:

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

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)

2. Tissue or cell sample:

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

11. Performance Characteristics

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

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:

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

$$= 0.43\ \mu\text{ mol/mL}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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