Technical Manual

Pyruvic Acid Colorimetric Assay Kit

- **Catalogue Code: MAES0104**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.003-2.0 μmol/mL

Sensitivity:

0.003 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.

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2. Background

Pyruvic acid is the simplest of the alpha-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 pyruvic acid content in serum, plasma and tissue samples.

4. Detection Principle

Pyruvic acid reacts with the chromogenic agent to produce a reaction product that 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	1.2 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	6 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Alkaline Reagent	20 mL × 1 vial	2-8°C, 6 months
Sodium Pyruvate Standard (2 μmol/mL)	1.6 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (480-520 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:

Bring all reagents to room temperature 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°C. Take the supernatant and preserve on ice before 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 (0.003-2.0 μmol/mL).

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

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);

8. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 505 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

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9. Operation Steps

The preparation of standard curve

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.

The measurement of 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 10s, then incubate at 37°C for 10 min.

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

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

The measurement of tissue sample

1. **Standard well:** Add 15 μL of standard solution with different concentrations, 10 μL of clarificant and 50 μL of chromogenic agent to the corresponding wells.
   Sample well: Add 15 μL of sample, 10 μL of clarificant and 50 μL of chromogenic agent to the corresponding wells.

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

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

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

Operation Table For Serum (Plasma)

	Standard well	Sample well
Standard solution with different concentrations (μL)	15	
Sample (μL)		15
Chromogenic agent (μL)	50	50
Mix fully with microplate reader for 10s, then incubate at 37°C for 10 min.		
Alkaline reagent (μL)	150	150
Mix fully with microplate reader for 10s, then incubate at room temperature for 5 min. Measure the OD value of each well at 505 nm with microplate reader.		

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Operation Table For Tissue

	Standard well	Sample well
Standard solution with different concentrations (µL)	15	
Sample (µL)		15
Clarificant (µL)	10	10
Chromogenic agent (µL)	50	50
Mix fully with microplate reader for 10s, then incubate at 37°C for 10 min.		
Alkaline reagent (µL)	150	150
Mix fully with microplate reader for 10s, then incubate at room temperature for 5 min. Measure the OD value of each well at 505 nm with microplate reader.		

10. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum/plasma samples:

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

2. Tissue samples:

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

y: $OD_{Standard} - OD_{Blank}$.

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before test.

ΔA_{505}: $OD_{Sample} - OD_{Blank}$.

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

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11. Performance Characteristics

Detection Range	0.003-2.0 µmol/mL
Sensitivity	0.003 µmol/mL
Average recovery rate (%)	95
Average inter-assay CV (%)	3.6
Average intra-assay CV (%)	2.3

Analysis

Take 15 µL of human serum sample, carry the assay according to the operation table.

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:

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

$$= 0.42 \ \mu mol/mL$$

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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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