TECHNICAL MANUAL

Pyruvic Acid Colorimetric Assay Kit

- **SKU CODE:** MAES0104
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate at 37°C for 10 min
- Add alkali reagent
- Incubate at room temperature for 5 min
- Measure OD at 505 nm

2. Intended use

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

3. Detection principle

Pyruvic acid reacts 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.

4. 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	2 pieces	

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **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 create 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.

7. Standard dilution table

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

8. Sample preparation

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 10,000×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 (MAES0177).

Sample dilution:

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

9. Sample dilution table

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

10. Operating steps

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.

11. 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:

Pyruvic Acid (μmol/mL) = (ΔA_505 - b) ÷ a × f

2. Tissue sample:

Pyruvic Acid (μmol/mgprot) = (ΔA_505 - b) ÷ a × f ÷ C_{pr}

[Note]

f: Dilution factor of sample before tested.

ΔA_505: OD_{Sample} - OD_{Blank}.

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

12. Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision Table

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.

Inter-assay Precision Table

Parameters	Sample 1	Sample 2	Sample 3
Mean (µmol/mL)	0.55	1.20	1.60
%CV	3.6	3.5	3.7

Recovery

Three samples of high, middle, and low concentration were tested with each concentration measured 6 times in parallel to obtain an average recovery rate of 95%.

Recovery Table

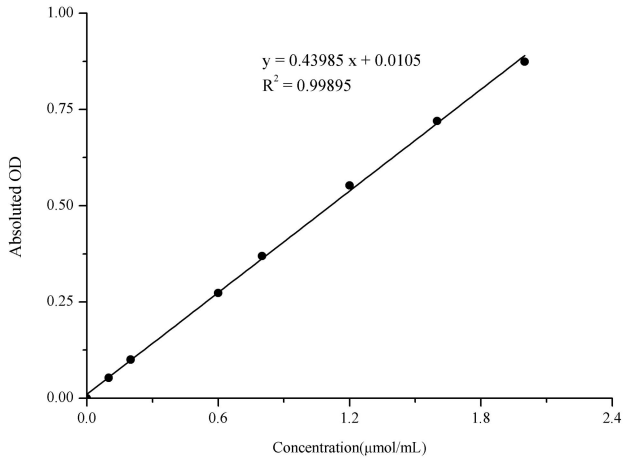
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/mL)	0.15	0.7	1.4
Observed Conc. (µmol/mL)	0.1	0.7	1.3
Recovery rate (%)	97	96	92

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.

Standard curve data

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), the standard curve and data are provided below for reference only:



Standard curve table

Concentration (µmol/mL)	OD value 1	OD value 2	Average OD	Absoluted OD
0.0	0.047	0.049	0.048	0.000
0.1	0.105	0.097	0.101	0.053
0.2	0.145	0.152	0.148	0.100
0.6	0.326	0.317	0.321	0.273
0.8	0.419	0.415	0.417	0.369
1.2	0.601	0.600	0.601	0.553
1.6	0.768	0.769	0.768	0.720
2.0	0.896	0.949	0.922	0.874

13. Example Analysis

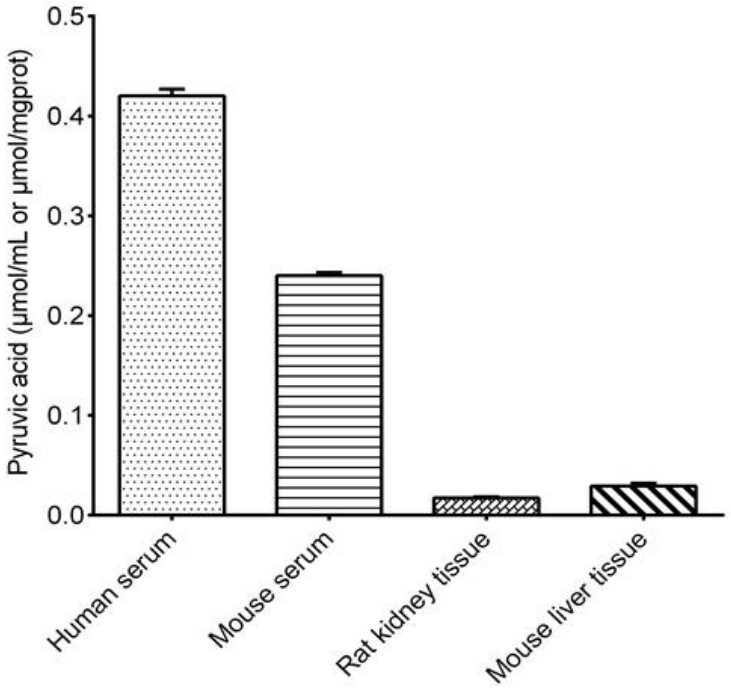
Example analysis:

Take 15 µL of human serum sample and carry out the assay according to the operating 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:

Pyruvic Acid (µmol/mL) = (0.269 - 0.047 - 0.0123) ÷ 0.4983 = 0.42 µmol/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:



14. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.