CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Cat #: KTB1121 Size: 48 T/48 S 96 T/96 S

	Micro Pyruvate Acid (PA) Assay Kit		
REF	**Cat #:** KTB1121	LOT	**Lot #:** Refer to product label
	Detection range: 1.094-70 μg/mL		**Sensitivity:** 0.5 μg/mL
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Pyruvate Acid (PA) connects the three major metabolisms of glucose, fatty acids and amino acids through acetyl CoA, and plays an important pivotal role. Abbkine CheKine™ Micro Pyruvate Acid (PA) Assay Kit is specially developed for the detection of Pyruvate Acid in a variety of biological samples such as Plasma, Serum, Animal and Plant Tissues, Cells and Bacteria. The operation is simple and convenient, and the detection is more sensitive and accurate. The Pyruvate Acid reacts with 2,4-dinitrophenylhydrazine to produce a red compound with a maximum absorption peak at 520 nm. Within a certain concentration range, the Pyruvate Acid content has a linear relationship with the absorbance at 520 nm. According to the standard curve, the Pyruvate Acid content in the sample can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Chromogen A	1.75 mL	3.5 mL	4℃, protected from light
Chromogen B	8.75 mL	17.5 mL	4℃
Standard (1 mg/mL)	1 mL	1 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 520 nm
- Incubator, ice maker, refrigerated centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

• Deionized water
• Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Chromogen A: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Note: Extraction Buffer is toxic and Chromogen A has a pungent odor, so it is recommended to experiment in a fume hood.
Chromogen B: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Standard preparation:
Standard curve setting: dilute 1 mg/mL Standard with Extraction Buffer to 70, 35, 17.5, 8.75, 4.375, 2.188, 1.094 μg/mL standard solution as shown in the table below.

Num.	Volume of Standard	Volume of Extraction Buffer (μL)	The Concentration of Standard (μg/mL)
Std.1	35 μL 1 mg/mL	465	70
Std.2	200 μL of Std.1 (70 μg/mL)	200	35
Std.3	200 μL of Std.2 (35 μg/mL)	200	17.5
Std.4	200 μL of Std.3 (17.5 μg/mL)	200	8.75
Std.5	200 μL of Std.4 (8.75 μg/mL)	200	4.375
Std.6	200 μL of Std.5 (4.375 μg/mL)	200	2.188
Std.7	200 μL of Std.6 (2.188 μg/mL)	200	1.094

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.
1. Animal tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize, letting stand for 30 min. Centrifuge at 4,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
2. Plant tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize, then ultrasonically disrupt the plant tissue 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times), letting stand for 30 min. Centrifuge at 4,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
3. Cells or bacteria: Collect 5×10^6 bacteria or cells into the centrifuge tube, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria or cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times), letting stand for 30 min. Centrifuge at 4,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
4. Plasma, serum: Take 100 μL plasma (serum), add 1 mL Extraction Buffer, mix well, letting stand for 30 min. Centrifuge at 4,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 520 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)

Sample	0	0	75
Different Concentration of Std.	0	75	0
Extraction Buffer	75	0	0
Chromogen A	25	25	25
Mix well, letting stand for 2 min at room temperature			
Chromogen B	125	125	125

3. Mix well, then reading the values at 520 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$. (Only one blank well needs to be detected)

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the Standard Solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (μg/mL).

2. Calculate the content of Pyruvate Acid in sample

(1) By sample fresh weight

Pyruvate Acid (μg/g)=$(y\times V_{Sample})\div(W\times V_{Sample}\div V_{Extraction\ Buffer})\times n$**=y÷W×n**

(2) By liquid volume of plasma (serum)

Pyruvate Acid (μg/mL)=$(y\times V_{Sample})\div(V_{Liquid}\times V_{Sample}\div V_{Extraction\ Buffer})\times n$**=10×y×n**

(3) By number of cells or bacteria

Pyruvate Acid (μg/10^4)=$(y\times V_{Sample})\div(500\times V_{Sample}\div V_{Extraction\ Buffer})\times n$**=y÷500×n**

Where: V_{Sample}: the volume of add sample volume (0.075 mL); $V_{Extraction\ Buffer}$: the volume of add Extraction Buffer (1 mL); W: the weight of sample (g); V_{Liquid}: the liquid sample volume of be taken (0.1 mL); 500: the number of cells or bacteria, 5×10^6; n: the the sample dilution factor.

Typical Data

Typical standard curve:

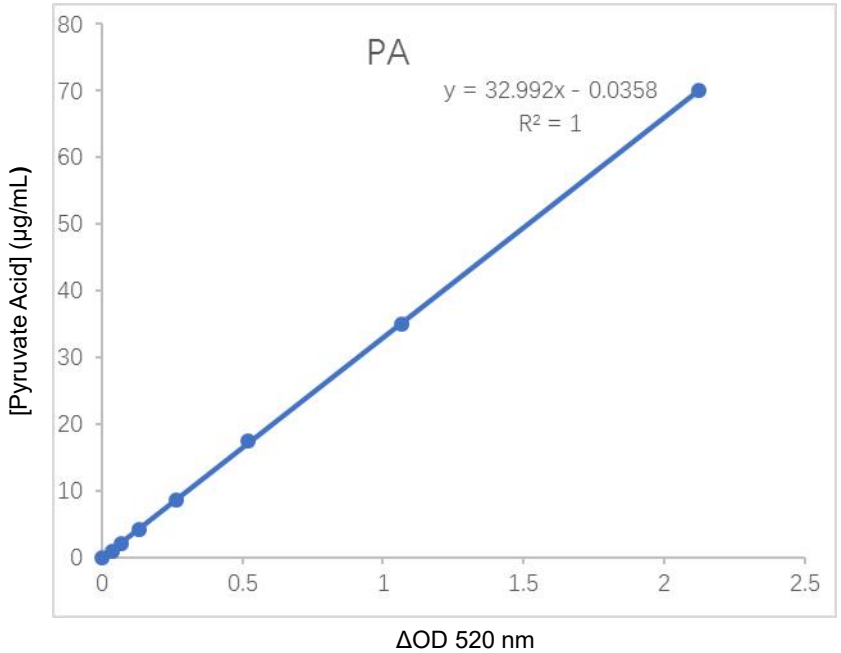

Figure 1. Standard curve of Pyruvate Acid in 96-well plate assay-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Examples:

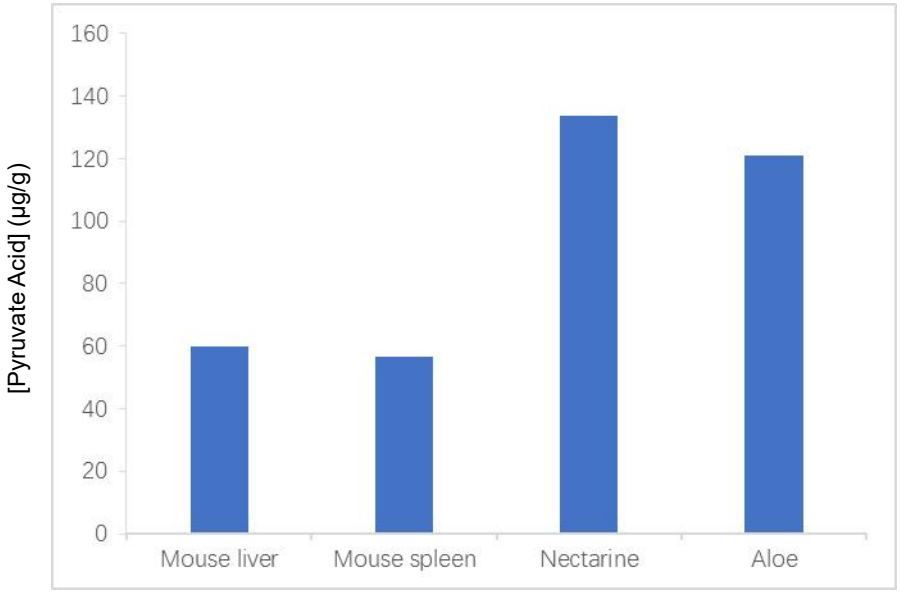

Figure 2. Pyruvate Acid concentration in mouse liver, mouse spleen, nectarine and aloe respectively. Assays were performed following kit protocol.

Recommended Products

Catalog No.	Product Name
KTB1120	CheKine™ Micro Pyruvate Kinase (PK) Activity Assay Kit
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Assay Kit
KTB1122	CheKine™ Micro Phosphoenolpyruvate Carboxylase (PEPC) Activity Assay Kit
KTB1100	CheKine™ Micro Lactate Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.