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### CheKine™ Micro Soil Pyruvate (S-PA) Assay Kit

Cat #: KTB4061

Size: 48 T/48 S 96 T/96 S

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REF	<b>Cat #</b> : KTB4061	LOT	Lot #: Refer to product label
	Detection range: 1.094-70 µg/mL		Sensitivity: 0.5 μg/mL
	Applicable samples: Soil		
X	Storage: Stored at 4°C for 6 months, protected from light		

### **Assay Principle**

Pyruvate (PA) connects the three major metabolisms of glucose, fatty acids and amino acids through acetyl CoA, and plays an important pivotal role. Abbkine CheKine<sup>™</sup> Micro Soil Pyruvate (S-PA) Assay Kit is specially developed for the detection of Pyruvate in a variety of biological samples such as soil. The operation is simple and convenient, and the detection is more sensitive and accurate. The Pyruvate 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 content has a linear relationship with the absorbance at 520 nm. According to the standard curve, the Pyruvate content in the sample can be calculated.

### Materials Supplied and Storage Conditions

	Si	ze	Storege conditions	
Kit components	48 T	96 T	Storage conditions	
Extraction Buffer	70 mL	70 mL×2	4°C, protected from light	
Reagent	1.8 mL	3.6 mL	4°C, protected from light	
Reagent II	8.8 mL	17.6 mL	4°C	
Standard (1 mg/mL)	1 mL	1 mL	4°C	

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, centrifuge, Ultrasonic cleaner, 30-50 mesh sieve
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Deionized water



## **Reagent Preparation**

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C., protected from light. Reagent I : Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light. Note: Extraction Buffer is toxic and Reagent I has a pungent odor, so it is recommended to experiment in a fume hood.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

#### 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: It is recommended to use fresh soil samples.

Fresh soil samples naturally air dried or air dried in an oven at 37°C and sieved through 30-50 mesh sieve. Weigh 0.1 g dried soil, add 1 mL Extraction Buffer and homogenize, sonic in ultrasonic cleaner for 30 min at room temperature. Centrifuge at 12,000 g for 10 min at room temperature. Use supernatant for assay.

### **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
Reagent	25	25	25
Mix well, letting stand for 2 min at room temperature			
Reagent II	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)

Note: The Standard Well and Blank Well only need to be done once or twice. In order to guarantee the accuracy of



experimental results, need to do a pre-experiment with 2-3 samples. If  $\Delta A_{Test}$  is less than 0.01, increase the sample quantity appropriately. If  $\Delta A_{Test}$  is greater than  $\Delta A_{Standard}$  of 70 µg/mL, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

## **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 x-axis and the  $\Delta A_{Standard}$  as the y-axis, draw the standard curve and obtain the standard equation. The determination of  $\Delta A_{Test}$  is substituted into the equation to get x (µg/mL).

2. Calculate the content of S-PA in soil

 $S\text{-PA} (\mu g/g \text{ soil}) = (x \times V_{Sample}) \div (W \times V_{Sample} \div V_{Extraction Buffer}) = x \div W$ 

Where: V<sub>Sample</sub>: the volume of added sample volume, 0.075 mL; V<sub>Extraction Buffer</sub>: the volume of added Extraction Buffer, 1 mL; W: the weight of sample, g.

# **Typical Data**

1 0.9 0.8 0.7 AStandard 0.6 y = 0.0135x + 0.0031R<sup>2</sup> = 0.9998 0.5 0.4 0.3 0.2 0.1 0 0 10 20 30 40 50 60 70 80 Standard content (µg/mL)

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.





Figure 2. Determination of S-PA in soil by this kit.

# **Recommended Products**

Catalog No.	Product Name



KTB4012	CheKine™ Micro Soil Nitrate Nitrogen Assay Kit
KTB4014	CheKine™ Micro Acid Soil Available Phosphorous Assay Kit
KTB4041	CheKine™ Micro Soil Alkaline Phosphatase (S-AKP/ALP) Activity Assay Kit
KTB4050	CheKine™ Micro Soil Catalase (S-CAT) Activity 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.

