

## Soil Pyruvate (S-PA) Content Assay kit (enzymatic method)

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/microplate reader

**Cat No:** BC5535

**Size:** 100T/48S

### Components:

**Reagent I:** Liquid 25 mL×1, Storage at 2-8°C;

**Reagent II:** Powder×1, Storage at -20°C. Before use, add 3.6mL distilled water to dissolve the powder thoroughly. Unused reagents can be stored in aliquots at -20°C for 4 weeks, avoiding repeated freezing and thawing;

**Reagent III:** Liquid 13 μL×1, Storage at 2-8°C; **Mix Reagent III:** distilled water = 1μL: 100μL (101 μL, about 6T) according to the dosage before use, and prepare for use now.

**Reagent IV:** Liquid 4 mL×1, Storage at 2-8°C;

**Standard solution:** Liquid 1 mL×1, Storage at 2-8°C; 20μmol/mL Standard solution of sodium pyruvate.

### Product Description:

Pyruvate plays an important role in the metabolism of glucose, fatty acids and amino acids through acetyl CoA.

At pH=7.5, pyruvate reacts with NADH catalyzed by LDH to produce NAD<sup>+</sup> and lactic acid. Under 1-mPMS, WST-1 reacts with NADH to produce water-soluble Formazan. Pyruvate content can be calculated by detecting the absorption value at 450nm.

### Required but Not Provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, water-bath/ constant temperature incubator, adjustable pipette, micro quartz cuvette/96 well flat-bottom plate, ultrasonic cleaning instrument, 30-50 mesh sieve, ice and distilled water.

### Protocol

#### I. Preparation:

1. Fresh soil sample natural air dry or 37°C oven air dry, 30~50 mesh sieve.
2. Weigh the air-dried mixed soil sample about 0.1g, add 1mL distilled water and mix; Then put the ultrasonic cleaning instrument in room temperature ultrasonic 30minutes. Then 12000g was centrifuged at room temperature for 10minutes, and the supernatant was taken to be measured.

#### II. Determination procedure:

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 450 nm, set the counter to zero with distilled water.
2. Preheat Reagent I in 37°C for 20 minutes.

3. Standard tube measurement: 20 $\mu$ mol/mL sodium pyruvate standard solution was diluted with distilled water to obtain 0.5, 0.25, 0.125, 0.0625, 0.03125 $\mu$ mol/mL standard solution for reserve.

4. Standard dilution table

Number	Predilution concentration ( $\mu$ mol/mL)	Standard liquid volume ( $\mu$ L)	distilled water volume ( $\mu$ L)	Diluted concentration ( $\mu$ mol/mL)
1	20	50	950	1
2	1	625	375	0.625
3	0.625	800	200	0.5
4	0.5	625	375	0.3125
5	0.3125	800	200	0.25
6	0.25	625	375	0.15625
7	0.15625	800	200	0.125

Remarks: 20 $\mu$ L of standard solution was required for each standard tube.

5. Operation table:

Reagent Name ( $\mu$ L)	Test tube (A <sub>T</sub> )	Control tube (A <sub>C</sub> )	Standard tube (A <sub>S</sub> )	Blank tube1 (A <sub>B1</sub> )	Blank tube2 (A <sub>B2</sub> )
Sample	20	20	-	-	-
Standard solution	-	-	20	-	-
distilled water	-	-	-	20	<b>40</b>
Reagent I	155	<b>180</b>	155	155	155
Reagent II	10	-	10	10	10
Reagent III	15	-	15	15	15
After mixing, react at 37°C for 30min					
Reagent IV	20	20	20	20	-

After mixing, reaction at 37°C for 30minutes (avoiding light). Absorb 200 $\mu$ L in micro quartz cuvette/96 well flat-bottom plate, measure the absorbance at 450nm, calculate  $\Delta A_T = (A_{B1} - A_{B2}) - (A_T - A_C)$ ,  $\Delta A_S = A_{B1} - A_S$ . (Standard curve, blank tube 1 and blank tube 2 only need to be measured 1-2 times).

### III. III. Calculation of pyruvate content in soil:

1. Standard curve drawing:

According to the concentration of the standard tube (x,  $\mu$ mol/mL) and the absorbance  $\Delta A_S$  (y,  $\Delta A_S$ ), the standard curve was established. According to the standard curve, the determination of  $\Delta A$  is substituted into the equation to obtain x ( $\mu$ mol/mL).

2. Soil pyruvate content calculation:

$$S\text{-PA } (\mu\text{mol/g weight}) = x \times V_S \div (V_S \div V_E \times W) = x \div W$$

$V_S$ : added sample volume, 0.1mL;

$V_E$ : The volume of distilled water added in pre-treatment, 1mL;

W: Sample mass, g.

**Note:**

1. If the absorbance value exceeds the linear range, the sample size can be increased or diluted with distilled water before the determination.

**Experimental example:**

1. Take 0.103g Flowerpot soil, add 1mL distilled water, ultrasonic crushing 30minutes, 10000g, centrifugal at room temperature 10minutes, take supernatant to be measured. operate according to the measurement steps, use 96 well flat-bottom plate to measure  $\Delta A_T = (A_{B1} - A_{B2}) - (A_T - A_C) = (0.601 - 0.048) - (0.455 - 0.067) = 0.165$ , Standard curve  $y = 0.8017x - 0.0471$ ,  $R^2 = 0.9985$ ,  $x = 0.265 \mu\text{mol/mL}$ , pyruvate content calculated:

$$\text{S-PA content } (\mu\text{mol /g weight}) = x \times V_S \div (V_S \div V_E \times W) = x \div W = 2.573 \mu\text{mol /g weight}$$

2. Take 0.101g sludge, add 1mL distilled water, ultrasonic crushing 30minutes, 10000g, centrifugal at room temperature 10minutes, take supernatant to be measured. operate according to the measurement steps, use 1mL glass cuvette to measure  $\Delta A_T = (A_{B1} - A_{B2}) - (A_T - A_C) = (0.601 - 0.048) - (0.331 - 0.07) = 0.292$ , Standard curve  $y = 0.8017x - 0.0471$ ,  $R^2 = 0.9985$ ,  $x = 0.423 \mu\text{mol/mL}$ , pyruvate content calculated:

$$\text{S-PA content } (\mu\text{mol /g weight}) = x \times V_S \div (V_S \div V_E \times W) = x \div W = 4.188 \mu\text{mol /g weight}$$

**Related Products:**

BC5260/BC5265	Pyruvate (PA) Content Assay Kit (Enzymatic Method)
BC0380/BC0385	Pyruvate Dehydrogenase (PDH) Activity Assay Kit
BC0710/BC0715	$\alpha$ -Ketoglutarate Dehydrogenase ( $\alpha$ -KGDH) Activity Assay Kit
BC0950/BC0955	Succinate Dehydrogenase (SDH) Activity Assay Kit

