

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

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

Operation Equipment: Spectrophotometer

Cat No: BC5530

Size: 50T/24S

Components:

Reagent I: Liquid 60 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 40 μL×1, Storage at 2-8°C; **Mix Reagent III:** distilled water = 10μL: 1mL (1.01 mL, about 13T) according to the dosage before use, and prepare for use now.

Reagent IV: Liquid 8 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⁺ 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, desk centrifuge, water-bath/ constant temperature incubator, adjustable pipette, ultrasonic cleaning instrument, 30-50 mesh sieve, 1 mL glass cuvette, 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 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: 20μmol/mL sodium pyruvate standard solution was diluted with

distilled water to obtain 0.5, 0.25, 0.125, 0.0625, 0.03125 $\mu\text{mol/mL}$ standard solution for reserve.

4. Standard dilution table

Number	Predilution concentration ($\mu\text{mol/mL}$)	Standard liquid volume (μL)	distilled water volume (μL)	Diluted concentration ($\mu\text{mol/mL}$)
1	20	50	950	1
2	1	500	500	0.5
3	0.5	500	500	0.25
4	0.25	500	500	0.125
5	0.125	500	500	0.0625
6	0.0625	500	500	0.03125

Remarks: 100 μL of standard solution was required for each standard tube.

5. Operation table: (The following reagents were added to a 1.5mLEP tube)

Reagent Name (μL)	Test tube (A_T)	Control tube (A_C)	Standard tube (A_S)	Blank tube (A_B)
Sample	100	100	-	-
Standard solution	-	-	100	-
distilled water	-	-	-	100
Reagent I	775	900	775	775
Reagent II	50	-	50	50
Reagent III	75	-	75	75
After mixing, react at 37°C for 30min				
Reagent IV	100	100	100	100
After mixing, reaction at 37°C for 30minutes (avoiding light). The absorbance at 450nm was measured in A 1mL glass colorimetric dish, and $\Delta A_T = A_B - (A_T - A_C)$, $\Delta A_S = A_B - A_S$. (Standard curve and blank tube only need to be measured 1-2 times).				

III. Calculation of pyruvate content in soil:

1. Standard curve drawing:

According to the concentration of the standard tube (x, $\mu\text{mol/mL}$) and the absorbance ΔA_S (y, ΔA_S), the standard curve was established. According to the standard curve, the determination of ΔA is substituted into the equation to obtain x ($\mu\text{mol/mL}$).

2. Soil pyruvate content calculation:

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

V_S : added sample volume, 0.1mL;

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

W: Sample mass, g.

Note:

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

Experimental example:

- 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 1mL glass cuvette to measure $\Delta A_T = A_B - (A_T - A_C) = 0.843 - (0.493 - 0.028) = 0.378$, Standard curve $y = 1.4576x - 0.0002$, $R^2 = 0.9924$, $x = 0.2595 \mu\text{mol/mL}$, pyruvate content calculated:

$$\text{S-PA content } (\mu\text{mol /g weight}) = x \times V_S \div (V_S \div V_E \times W) = x \div W = 2.5191 \mu\text{mol /g weight}$$

- 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 $\Delta A_T = A_B - (A_T - A_C) = 0.843 - (0.528 - 0.135) = 0.450$, Standard curve $y = 1.4576x - 0.0002$, $R^2 = 0.9924$, $x = 0.3089 \mu\text{mol/mL}$, pyruvate content calculated:

$$\text{S-PA content } (\mu\text{mol /g weight}) = 0.02 \times \Delta A \div \Delta A_s \div W \times F = 3.0581 \mu\text{mol /g weight}$$

Related Products:

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

