CheKine™ Micro Soil Polyphenol Oxidase (S-PPO) Activity Assay Kit

Cat #: KTB4029 Size: 48 T/48 S 96 T/96 S

	Micro Soil Polyphenol Oxidase (S-PPO) Activity Assay Kit		
REF	**Cat #:** KTB4029	LOT	**Lot #:** Refer to product label
	Applicable samples: Soil sample		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Soil Polyphenol Oxidase (S-PPO) primarily originates from soil microorganisms, plant root exudates, and the decomposition of animal and plant residues. It catalyzes the oxidation of aromatic compounds in the soil into quinones. These quinones then react with proteins, amino acids, sugars, minerals, and other substances in the soil to form organic matter and pigments, completing the cycle of aromatic compounds in the soil. This process is crucial for soil environmental remediation. CheKine™ Soil Polyphenol Oxidase (S-PPO) Activity Assay Kit provides a simple, convenient, and rapid method for detecting S-PPO activity in soil samples. The principle of this assay is based on the ability of S-PPO to catalyze the oxidation of pyrogallol (1,2,3-benzenetriol) to produce a colored product, which exhibits characteristic light absorption at 430 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Reagent Ⅱ	3.5 mL	7 mL	4℃
Standard	2 mL	2 mL	4℃, protected from light

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 430 nm
- 96-well quartz/glass plate (non-polystyrene material) or microglass cuvette, precision pipettes, disposable pipette tips
- Oven, 30-50 mesh sieve, centrifuge, constant temperature water bath, analytical balance
- Deionized water, ether, 0.5 mol/L HCl solution

Reagent Preparation

Reagent Ⅰ : Prepare fresh before use. Take one bottle of Reagent Ⅰ and add 9 mL of deionized water, ensuring thorough

dissolution by mixing well. The prepared reagent is then ready for use. Any unused reagent should be stored at 4℃ and can be kept for up to one week.

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

Standard: A 10 mmol/L potassium dichromate solution is equivalent to a 0.4 mg/mL solution of purple pyrogallol.

Note: Reagent Ⅰ and Standard are toxic, so it is recommended to experiment in a fume hood.

Standard Curve Setting: According to the table provided, dilute the Standard to the following concentrations using 0.5 mol/L HCl solution: 0.2, 0.1, 0.05, 0.025, 0.0125 and 0.00625 mg/mL.

Num.	Volume of Standard	Volume of 0.5 mol/L HCl (μL)	The Concentration of Standard (mg/mL)
Std.1	600 μL of Standard	0	0.4
Std.2	300 μL of Std.1 (0.4 mg/mL)	300	0.2
Std.3	300 μL of Std.2 (0.2 mg/mL)	300	0.1
Std.4	300 μL of Std.3 (0.1 mg/mL)	300	0.05
Std.5	300 μL of Std.4 (0.05 mg/mL)	300	0.025
Std.6	300 μL of Std.5 (0.025 mg/mL)	300	0.0125
Std.7	300 μL of Std.6 (0.0125 mg/mL)	300	0.00625
Blank	0	300	0 (Blank Well)

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.

Fresh soil samples should be air-dried naturally or dried in an oven at 37℃, then passed through a 30-50 mesh sieve.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 430 nm, visible spectrophotometer was returned to zero with deionized water.
2. Establishment of the Standard Curve: Use the 0 mg/mL standard as the blank control. Take 200 μL of each diluted standard (0.4, 0.2, 0.1, 0.05, 0.025, 0.0125, and 0.00625 mg/mL) and transfer them to a 96-well quartz/glass plate (non-polystyrene material) or micro glass cuvette. Measure the absorbance (A) at 430 nm. Record the absorbance values as $A_{Standard}$ and A_{Blank}. The standard curve needs to be performed only 1-2 times.
3. Sample Measurement (The following operations are performed in 1.5 mL centrifuge tubes):

Reagent	Test Tube（μL）
Air-dried soil sample (g)	0.02
Reagent Ⅰ	120
Mix by shaking and incubate at a constant temperature of 30℃ for 1 h	
Reagent Ⅱ	50
Ether	430

Mix by shaking several times and let the mixture stand at room temperature for 30 min. Transfer 200 μL of the supernatant to a 96-well quartz/glass plate (non-polystyrene material) or a micro glass cuvette. Measure the absorbance (A) at 430 nm, which was recorded as A_{Test}, calculated $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: 1. Because of the low viscosity of ether, it tends to drip easily. Before aspirating, rinse the pipette tip 2-3 times with the supernatant to ensure accurate transfer for subsequent measurements. 2. Ether is highly volatile. After transferring the sample to a 96-well quartz/glass plate (non-polystyrene material) or a micro glass cuvette, measure the absorbance immediately. It is recommended to measure each sample individually to minimize evaporation. 3. The standard curve needs to be determined only once, and a control well should be set up for each measurement well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.5, dilute the sample appropriately before proceeding with the measurement, and adjust the calculation formula accordingly.

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. Substitute the ΔA_{Test} into the equation to obtain the x value (mg/mL).

2. Calculation of S-PPO activity

Definition of unit: One enzyme activity unit is defined as the production of 1 mg of purple pyrogallol per g of soil sample per day.

S-PPO (U/g soil)=$x \times V_{Total\ reaction} \div W \div T$**$=10.32 \times x \div W$**

Where: $V_{Total\ reaction}$: Volume of ether added for extraction, 0.43 mL; W: Sample mass, 0.02 g, T: Reaction time, 1/24 d.

Typical Data

Typical standard curve:

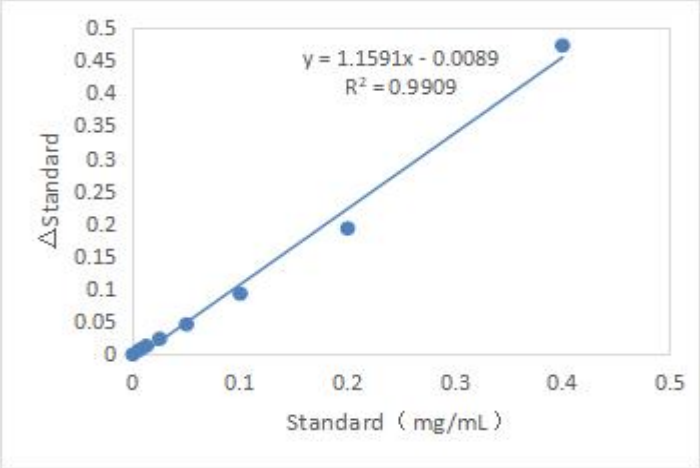

Figure1. Standard Curve for purple pyrogallol.

Examples:

Take 0.02 g of fresh soil sample that has been dried in a 37℃ oven and use 96-well plate (non-polystyrene material) to calculate ΔA_{Test}=0.128-0.036=0.092, x=0.087. The content calculated according to the soil sample mass is as follows:

S-PPO (U/g soil)=10.32×0.087÷0.02=44.892 U/g.

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.