



CheKine™ Micro Soil Peroxidase (S-POD) Activity Assay Kit

Cat #: KTB4023

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

	Micro Soil Peroxidase (S-POD) Activity Assay Kit		
REF	Cat #: KTB4023	LOT	Lot #: Refer to product label
	Applicable samples: Soli		
	Storage: Stored at 4°C for 6 months, protected from light		

Assay Principle

S-POD mainly comes from soil microorganism, which can oxidize organic matter in soil to produce peroxide, which plays an important role in the process of humus formation. CheKine™ Micro Soil Peroxidase (S-POD) Activity Assay Kit can be used to detect biological samples such as soli. In the kit, S-POD catalyze the oxidation of organic substances to quinone which has an 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°C, protected from light
Reagent II	1.25 mL	2.5 mL	4°C
Reagent III	6 mL	12 mL	4°C
Reagent IV	4 mL	4 mL	4°C
Standard	2 mL	2 mL	4°C, 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 plate/glass plate (non-polystyrene /polypropylene)** or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, centrifuge, 30-50 mesh sieve
- Deionized water, Ether

Reagent Preparation

Working Reagent I: Prepared before use. Add 12 mL deionized water to each bottle, fully dissolve; The remaining reagent can

also be stored at 4°C, protected from light for 1 week.

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

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

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

Standard : Ready to use as supplied; 10 mmol/L potassium dichromate solution, equivalent to 0.4 mg/mL purple gallic acid solution. Equilibrate to room temperature before use; Store at 4°C, protected from light.

Note: Reagent I, Reagent IV or Standard has certain irritation, so personal protection is recommended during use.

0.2mg /mL Standard: prepared before use; Take 150 µL Standard, add 150 µL Reagent IV, and mix thoroughly. The concentration is 5 mmol/L potassium dichromate solution, equivalent to 0.2 mg/mL purple gallic acid solution.

Note: 0.2 mg/mL Standard needs to be prepared for each experiment, and the diluted 0.2 mg/mL Standard should be used up within 4 h.

Sample Preparation

Note: 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.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 430 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Standard Well and Blank Well determination: Take Reagent IV as Blank Well. Take 200 µL Reagent IV and 200 µL 0.2mg/mL standard to micro glass cuvette/96-well plate respectively, detect the absorbance at 430 nm. The Blank Well is recorded as A_{Blank} , the Standard Well is marked as A_{Standard} . Finally calculate $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.
3. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Test Tube	Control Tube
Sample (g)	0.04	0.04
Working Reagent I (µL)	100	100
Reagent II (µL)	20	0
Deionized Water (µL)	0	20
Mix thoroughly, incubate at 37°C for 1 h.		
Reagent III (µL)	50	50
Ether (µL)	430	430

4. Shake for 2 min, centrifuge at 5,000 g for 10 min at 25°C. Add 200 µL to **96-well quartz plate / glass plate (non-polystyrene /polypropylene)** or microglass cuvette, detect the absorbance at 430 nm. The Test Well is marked as A_{Test} , and the Control Well is marked as A_{Control} . Finally calculate $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Control}}$.

Note: (1) The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. (2) If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.6, decrease the sample quantity appropriately. (3) Ether has irritation and corrosivity, so personal protection is recommended during use, and **96-well quartz plate / glass plate (non-polystyrene /polypropylene) or microglass cuvette. Because of the low viscosity of ether, it is easy to drop the liquid. Before absorption, it is necessary to moisten the gun head in the supernatant for 2-3 times, and then transfer it for determination. Ether is volatile, and it should be determined immediately after being transferred to **96-well quartz plate / glass plate (non-polystyrene /polypropylene)** or microglass cuvette, preferably one by one.**

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.

Calculation of the S-POD activity

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes the generation of 1 mg purple gallic acid in the reaction system every g sample everyday.

$$\text{S-POD(U/g soil)} = C_{\text{Standard}} \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}} \times V_{\text{Total volume}} \div W \div T = \mathbf{2.88 \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}} \div W}$$

C_{Standard} : 0.2 mg/mL purple gallic acid solution; $V_{\text{Total volume}}$: Reaction total volume, 0.6 mL; W : Sample weight, g; T : The reaction time, 1 h=1/24 d.

Typical Data

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

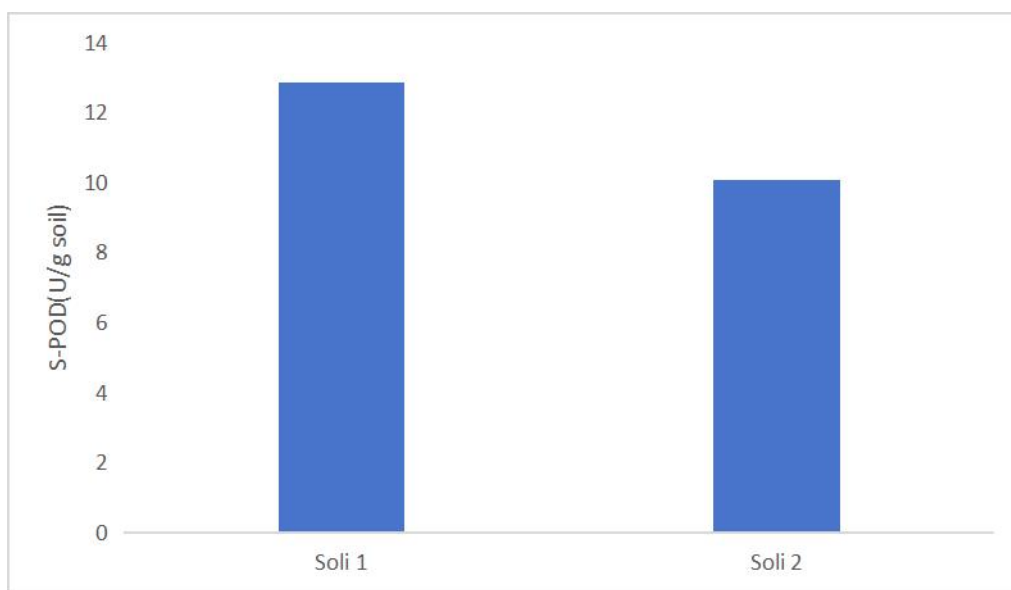


Figure 1. Determination of S-POD activity in soli sample by this kit.

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.