

## Soil Peroxidase(S-POD) Activity Assay Kit

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC0890

**Size:**50T/24S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Powder ×2	2-8°C
Reagent II	Liquid 5 mL×1	2-8°C
Reagent III	Liquid 10 mL×1	2-8°C
Reagent IV	Liquid 100 mL×1(required but not provided)	2-8°C
Standard	Liquid 10 mL×1	2-8°C

### Solution Preparation:

1. Reagent I: Add 10 mL of distilled water to one bottle of Reagent I. The left reagent could be stored at 2-8°C for one week.
2. Reagent IV: Ethyl ether. Required but not provided.
3. Standard: Equivalent to 0.2 mg/mL purple gallnut in per milliliter of ethyl ether.

### Product Description:

S-POD is mainly derived from soil microorganisms, which can oxidize organic matter in soil to produce peroxide, which plays an important role in the process of humus formation. S-POD catalyze the oxidation of organic substances to quinones which has an absorption peak at 430 nm.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer, desk centrifuge, water bath/constant temperature incubator, adjustable pipette, 1 mL glass cuvette, mortar, 30-50 mesh sieve, 0.5mol/L hydrochloric acid solution, ethyl ether (>98%, AR), ice and distilled water.

### Procedure:

#### I. Sample preparation:

Fresh soil sample: Air-drying or drying at 37°C, then passing through a 30-50 mesh sieve.

#### II. Determination:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 430 nm.
2. Standards preparation: Dilute the standard with **0.5mol/L hydrochloric acid solution** to 0.1, 0.08, 0.06, 0.04, 0.02, 0.01, 0 mg/mL.
3. Standards determination: set zero with **0 mg/mL standard**, detect the absorbance of each standard tube at 430nm, record as  $A_s$ .

## 4. Add reagents with the following list:

Reagent ( $\mu\text{L}$ )	Test tube ( $A_T$ )	Contrast tube ( $A_C$ )
Air-dried soil sample(g)	0.05	0.05
Distilled water	-	100
Reagent I	400	400
Reagent II	100	-
Shake to mix thoroughly, incubate at 30°C for 1 hour.		
Reagent III	200	200
Reagent IV	1750	1750
Mix thoroughly, place at room temperature for 30 minutes, set zero with <b>Reagent IV</b> . Take 1 mL of supernatant to detect the absorbance at 430nm, record as $A_T$ , $A_C$ . Calculate $\Delta A_T = A_T - A_C$ . A contrast tube is required for each test tube.		

**III. Calculation:**

## 1. Standard curve

The concentration of standard solution as y-axis ( $y$ , mg/mL),  $A_s$  ( $x$ ,  $A_s$ ) as x-axis, obtain the equation  $y=kx+b$ . Take  $\Delta A_T$  ( $x$ ) to the equation to acquire  $y$  value (mg/mL).

## 2. Calculation

Unit definition: One unit of soil peroxidase activity is defined as the amount enzyme catalyzes the generation of 1 mg of purple gallnut every gram of soil sample per day.

$$\text{S-POD activity (U /g soil sample)} = y \times V \div W \div T = 840 \times y$$

V: The total volume of Extract solution, 1.75 mL;

T: Reaction time, 1 hour = 1/24 day;

W: Sample weight, 0.05 g.

**Note:**

A contrast tube is required for each test tube.

**Recent Product Citations:**

- [1] Sun L, Zhang J, Zhao J, Lu X, Xiao C, Xiao Z, Zhang T, Gu Y, Sun H, Liu H, Li Y. Effects of Cinnamomum camphora coppice planting on soil fertility, microbial community structure and enzyme activity in subtropical China. *Front Microbiol.* 2023 Feb 3; 14:1104077. doi: 10.3389/fmicb.2023.1104077. PMID: 36819046; PMCID: PMC9936984.
- [2] Xiao J, Lan S, Farías ME, Qian L, Xia L, Song S, Wu L. The living forms of Microcoleus vaginatus and their contributions to the aggregate structure of biocrusts. *FEMS Microbiol Ecol.* 2023 Apr 7;99(5):fiad040. doi: 10.1093/femsec/fiad040. PMID: 37028939.

**References:**

[1] Doxey D L. The use of pyrogallol to demonstrate peroxidase in mammalian blood eosinophils[J]. Stain Technology, 1962, 37(6): 367-371.

[2] Nozaki O, Ji X, Kricka L J. New enhancers for the chemiluminescent peroxidase catalysed chemiluminescent oxidation of pyrogallol and purpurogallin[J]. Journal of bioluminescence and chemiluminescence, 1995, 10(3): 151-156.

**Related Products:**

- BC0110/BC0115 Soil Polyphenoloxidase (S-PPO) Activity Assay Kit
- BC0100/BC0105 Soil Catalase(S-CAT) Activity Assay Kit