

# Soil Polyphenol Oxidase (S-PPO) Activity Assay Kit

**Note:** The reagents of this product have changed, please pay attention to and strictly follow the instructions.

**Operation Equipment:** Microplate reader/Spectrophotometer

**Catalog Number:** BC0115

**Size:** 100T/48S

**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	Liquid 12 mL×1	2-8°C
Reagent II	Powder×2	2-8°C
Standard	Liquid 10 mL×1	2-8°C

## Solution Preparation:

1. Reagent II: Add 7 mL of distilled water to one Reagent I before use. Unused solution can be stored at 2-8°C for two weeks.
2. Standard: The potassium dichromate solution (5mmol/L) is equivalent to 0.2 mg/mL purple gallic acid solution.

## Product Description:

Soil polyphenol oxidase (S-PPO) mainly comes from the decomposition and release of soil microorganisms, plant root secretions as well as animals and plants residues. S-PPO catalyzes the oxidation of aromatic compounds into quinone in soil. Quinone reacts with proteins, amino acids, sugars, minerals and other substances in soil to generate organic matters and pigments. Therefore, S-PPO enables the soil to complete the cycle of aromatic compounds and be used for soil environmental restoration.

S-PPO can catalyze the pyrogallol to produce purple gallic acid, which has characteristic absorption peak at 430 nm.

**Note:** Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

## Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, water bath, adjustable pipette, micro glass cuvette/96 well plate (non-polystyrene), mortar, 30-50 mesh sieve, 0.5mol/L hydrochloric acid, ice and distilled water.

## Operation procedure:

**I. Sample preparation** (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

Fresh soil samples are naturally air-dried or oven to dry at 37°C, then sieved by 30- 50 mesh sieve.

## II. Determination procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 430 nm, set spectrophotometer to zero with distilled water.
2. Standard: Dilute the standard to 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 and 0 mg/mL standard with the 0.5 mol/L hydrochloric acid.
3. Standard dilution table

Serial number	The concentration before dilution(mg/mL)	Standard volume(μL)	Volume of 0.5 mol/L hydrochloric acid(μL)	Diluted concentration (mg/mL)
1	0.2	500	500	0.1
2	0.1	500	500	0.05
3	0.05	500	500	0.025
4	0.025	500	500	0.0125
5	0.0125	500	500	0.00625
6	0.00625	500	500	0.003125
7	-	0	500	0

**Note:** The following experiments require 200μL of standard for each standard tube.

4. Establishment of standard curve: Take 0 mg/mL standard product as blank tube, take 0.2 mL diluted standard into micro glass cuvette or 96 well plate, measure the absorbance value A at 430nm. Record as  $A_S$ ,  $A_B$ . Calculate  $\Delta A_S = A_S - A_B$ . The standard curve only need to be measured 1-2 times.
5. Sample determination: (add Reagent in the 1.5 mL tube)

Shake several times and stand at room temperature for 30 minutes, take 0.2 mL of supernatant to measure the absorbance value A at 430 nm.

Reagent	Test Tube(T)	Control Tube(C)
Air-dried soil sample(g)	0.04	0.04
Reagent I (μL)	100	100
Reagent II (μL)	240	-
distilled water (μL)		240

Fully mixed, reaction at 30°C for 1h, centrifugation at 5000g at 4°C for 10min, 200μL of supernatant was taken into micro glass cuvette or 96 well plate, measure the absorbance value A at 430nm. Record as  $A_T$ ,  $A_C$ . Calculate  $\Delta A_T = A_T - A_C$ . (Each test tube shall be provided with a control tube.)

## III. Calculation

## 1. Establishment of standard curve

According to the concentration (x, mg/mL) of the standard tube and the absorbance  $\Delta A_s$  (y,  $\Delta A_s$ ), establish a standard curve. According to the standard curve, bring  $\Delta A_T$  (y,  $\Delta A_T$ ) into the formula to calculate the sample concentration (x, mg/mL).

## 2. Calculation

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 mg of purple gallic acid per day every gram soil.

$$\text{S-PPO activity (U/g soil)} = x \times V_{ST} \div W \div T = 8.16 \times x \div W$$

T: Reaction time, 1 h=1/24 d;

$V_{ST}$ : Extract solution volume, 0.34 mL;

W: Sample quality, g.

### Note:

1. If the absorption value of the Test tube  $\Delta A_T > 1.2$ , the sample quality can be reduced for determination. If the absorption value of the Test tube  $\Delta A_T < 0.01$ , the sample quality can be increased for determination or the reaction time was extended and measured. Change the calculation formula simultaneously.
2. If the sample size is too large, it is recommended to test in batches. The supernatant after the reaction is placed on the ice to be measured, and the test is completed within 10min as far as possible to prevent the determination result from being affected by oxidation.

### Experimental example:

1. Weigh 0.04g Holly soil and follow the determination procedure. Using 96 well plate, measured  $\Delta A_T = A_T - A_C = 0.431 - 0.134 = 0.297$ . Bring it into the standard curve  $y = 5.8385x - 0.0164$  and calculate  $x = 0.054$ . Calculate S-PPO activity:

$$\text{S-PPO activity (U/g soil)} = 8.16 \times x \div W = 11.016 \text{ U/g soil}$$

### References:

[1] Montgomery M W, Sgarbieri V C. Isoenzymes of banana polyphenol oxidase[J]. Phytochemistry, 1975, 14(5-6): 1245-1249.

[2] Dogan S, Dogan M. Determination of kinetic properties of polyphenol oxidase from Thymus (Thymus longicaulis subsp. chaubardii var. chaubardii)[J]. Food chemistry, 2004, 88(1): 69-77.

### Related Products:

BC0100/BC0105 Soil Catalase(S-CAT) Activity Assay Kit

BC0120/BC0125 Soil Urease (S-UE) Activity Assay Kit

BC0890/BC0895 Soil Peroxidase(S-POD) Activity Assay Kit

BC5130/BC5135 Soil Superoxide Dismutase (S-SOD) Activity Assay Kit (WST Method)

BC5120/BC5125 Soil Malondialdehyde (S-MDA) Content Assay Kit