Solid-Polyphenol Oxidase(S-PPO) Activity Assay Kit

Product Code: 67074

Soil Polyphenol Oxidase (S-PPO) Activity Assay Kit - Microplate Method

Product Information

Product Code	67074
Assay Size	100T
Detection Method	Microplate method
Detection Wavelength	430 nm

Product Introduction

Soil polyphenol oxidase (S-PPO) is mainly derived from soil microorganisms, plant root exudates, and animal and plant residues released through decomposition. It catalyzes the oxidation of aromatic compounds in soil into quinones. These quinones react with soil proteins, amino acids, sugars, minerals, and other substances to generate organic matter and pigments, supporting the soil aromatic compound cycle and soil environmental remediation.

S-PPO catalyzes o-pyrogallol to produce purple purpurogallin, which has characteristic absorbance at 430 nm.

Package Contents

Item Code	Component	Quantity
67074.1	Reagent I	0.07 g × 2
67074.2	Reagent II	6 mL
67074.3	Standard	10 mL
67074.m	Instruction Manual	1 copy

Quality and Safety Information

Component	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Standard	--	--

Transportation and Storage

Transportation	Transport with ice packs.
Storage	Store at 2-8°C. Shelf life: 180 days.

Instructions for Use

1. Sample Processing

Naturally air-dry fresh soil samples, or dry them in a 37°C oven. Pass the dried samples through a 30-50 mesh sieve.

2. Reagent Preparation

1. Before use, add 7 mL double-distilled water to Reagent I. Store unused reagent at 4°C.

2. Prepare 50 mL ether.

3. Instrument Preparation

1. Preheat the microplate reader for at least 30 min.
2. Set the wavelength to 430 nm.
3. Zero the instrument with distilled water.

4. Standard Dilution

The standard is a 5 mmol/L potassium dichromate solution, equivalent to 0.2 mg/mL purpurogallin solution. Dilute the standard with 0.5 mol/L HCl solution to prepare 100, 50, 25, 12.5, 6.25, 3.125, and 0 μg/mL standard solutions for testing.

Serial No.	Concentration Before Dilution (μg/mL)	Standard Solution Volume (mL)	0.5 mol/L HCl Volume (mL)	Concentration After Dilution (μg/mL)
1	200	1.5	1.5	100
2	100	1.5	1.5	50
3	50	1.5	1.5	25
4	25	1.5	1.5	12.5
5	12.5	1.5	1.5	6.25
6	6.25	1.5	1.5	3.125
7	0	0	1.5	0

Each standard tube requires 0.2 mL standard solution. For this step, measure absorbance directly: add 0.2 mL standard to a micro glass cuvette or 96-well plate and measure at 430 nm. Record the absorbance as $A_{standard}$and A_{blank}. Calculate $\Delta A_{standard}$= $A_{standard}$- A_{blank}. The standard curve only needs to be measured 1-2 times.

5. Sample Assay Procedure

Component	Sample Tube	Blank Tube
Air-dried soil sample (g)	0.02	0.02
Reagent I (μL)	120	-
Distilled water (μL)	-	120
Shake and mix thoroughly, then incubate at 30°C for 1 h.		
Reagent II (μL)	50	50
Ether (μL)	430	430
Shake several times and let stand at room temperature for 30 min. Take 1 mL of the upper layer solution and measure the absorbance at 430 nm.		

Record the absorbance values as $A_{measurement}$and A_{blank}. Calculate Delta A = $A_{measurement}$- $A_{control}$.

Calculation

1. Standard Curve Preparation

Use the standard tube concentration (X, μg/mL) and the absorbance $\Delta A_{standard}$(Y) to establish the standard curve. Substitute the sample Delta A value (Y) into the standard curve formula to calculate the sample concentration (X, μg/mL).

2. S-PPO Activity Calculation

Unit definition: the production of 1 mg purpurogallin per g of soil sample per day is defined as one enzyme activity unit.

S-PPO activity (U/g soil sample) = $X \times V_{extraction} \div W \div T = 516 \times X$

Symbol	Description
T	Reaction time, 1 h = 1/24 d
$V_{extraction}$	Volume of ether added, 0.43 mL

W	Sample mass, 0.02 g

Precautions

1. This 100T kit can test 96 samples.
2. Because ether has low viscosity and droplets fall off easily, rinse the pipette tip in the upper layer solution 2-3 times before aspirating, then transfer for measurement.
3. Ether is volatile. After adding the upper solution to the cuvette, complete the absorbance measurement as soon as possible, preferably one sample at a time.
4. The linear range of this kit is 3.125-100 μg/mL.
5. Ether is corrosive to plastic, so glass 96-well plates are recommended.
6. Required instruments and supplies to be prepared by the user: microplate reader, water bath or metal bath, adjustable pipettes, glass 96-well plate, mortar, 30-50 mesh sieve, 0.5 mol/L HCl solution, ether, ice, and distilled water.

Appendix

For greater accuracy, prepare the standard curve before sample calculation. Follow the operating steps above. The provided standard curve formula may be used, or the absorbance values from each standard well may be used to prepare a standard curve with $R^2 \geq 0.99$ and obtain the calculation formula for samples.

Visual Reference