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

Product Code: 67072

Soil Polyphenol Oxidase (S-PPO) Activity Assay Kit

Product code: 67072

Method: Spectrophotometric method

Format: 50T

Product Introduction

Soil polyphenol oxidase (S-PPO) mainly originates from soil microorganisms, plant root exudates, and the decomposition and release of animal and plant residues. It catalyzes the oxidation of aromatic compounds in soil into quinones. Quinones then react with soil proteins, amino acids, sugars, minerals, and other substances to form organic matter and pigments, completing the cycle of soil aromatic compounds and supporting soil environmental remediation.

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

Example reference data for an air-dried soil sample: OD430 nm blank: 0.011; measurement: 0.289 / 0.287 / 0.282. Actual readings may vary depending on instruments and test conditions.

Package Contents and Storage

Code	Component	Quantity	Storage
67072.1	Reagent I	0.15 g × 2	Protect from light, 2–8°C
67072.2	Reagent II	12 mL	2–8°C
67072.3	Standard	10 mL	2–8°C
67072.m	Instructions	1 copy	Not applicable

Quality and Safety Information

Material	Quality Standard	Main Toxicity
Reagent I	Not specified	Not specified
Reagent II	Not specified	Not specified
Standard	Not specified	Not specified

Transportation and Storage

- **Transportation:** Shipped with ice packs.
- **Storage:** Store according to the package instructions.
- **Shelf life:** 180 days.

Instructions for Use

1. Sample Processing

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

2. Reagent Preparation

1. Before use, add 15 mL double-distilled water to each bottle of Reagent I. Store unused prepared reagent at 4°C.
2. Prepare 100 mL ether separately.

3. Instrument Preparation

1. Preheat the spectrophotometer for more than 30 min.
2. Set the wavelength to 430 nm.
3. Zero the instrument with distilled water.

4. Standard Dilution

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

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 1 mL standard solution. Transfer 1 mL standard solution to a 1 mL glass cuvette and measure absorbance at 430 nm. Record values as A standard and A blank. Calculate ΔA standard = A standard − A blank. The standard curve only needs to be measured 1–2 times.

5. Assay Procedure

Component or Step	Assay Tube	Blank Tube
Air-dried soil sample	0.05 g	0.05 g
Reagent I	500 μL	-
Distilled water	-	500 μL
Mixing and incubation	Shake and mix thoroughly, then incubate at 30°C for 1 h.	
Reagent II	200 μL	200 μL
Ether	1750 μL	1750 μL
Extraction and measurement	Shake several times and let stand at room temperature for 30 min. Transfer 1 mL of the upper-layer solution to a 1 mL glass cuvette and measure absorbance at 430 nm.	

Record the absorbance as A measurement and A blank. Calculate ΔA = A measurement − A control.

Calculation

1. Standard Curve

Use the standard tube concentration (X, μg/mL) and absorbance ΔA standard (Y, ΔA standard) to establish the standard curve. Substitute the sample ΔA value (Y, ΔA) into the standard curve formula to calculate the sample concentration (X, μg/mL).

2. S-PPO Activity

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

S-PPO activity (U/g soil sample) = X × V extraction ÷ W ÷ T = 840 × X

- X: sample concentration, μg/mL
- T: reaction time, 1 h = 1/24 d

- V extraction: volume of ether added, 1.75 mL
- W: sample mass, 0.05 g

Precautions

1. This 50T kit can test 48 samples.
2. Ether has low viscosity and drips easily. Before aspirating, rinse the pipette tip with the upper-layer liquid 2–3 times, then transfer for measurement.
3. Ether evaporates easily. After adding the upper-layer 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 a glass cuvette is recommended.

Required Materials Not Provided

- Visible spectrophotometer
- Water bath or metal bath
- Adjustable pipette
- 1 mL glass cuvette
- Mortar
- 30–50 mesh sieve
- 0.5 mol/L HCl solution
- Ether
- Ice
- Distilled water

Appendix

> For greater accuracy, prepare the standard curve during testing. Use the standard curve formula, or use the absorbance values of each standard obtained from the procedure table to plot a standard curve with $R^2 \geq 0.99$, then obtain the calculation formula for sample analysis.

Visual Reference