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

Product Code: 67073

Soil Polyphenol Oxidase Activity Assay Kit - Micromethod

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Product title: Solid-Polyphenol Oxidase (S-PPO) Activity Assay Kit

Product Introduction

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

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

Package Contents

Size	Code	Component	Quantity
100T	67073.1	Reagent I	0.07 g × 2
100T	67073.2	Reagent II	6 mL
100T	67073.3	Standard	10 mL
100T	67073.m	Manual	1 copy

Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent I	--	--
Reagent II	--	--
Standard	--	--

Shipping and Storage

Shipping	Shipped 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. User-provided reagent: 50 mL ether.

3. Operating Procedure

1. Preheat the spectrophotometer or microplate reader for at least 30 min. Set the wavelength to 430 nm and zero the instrument with distilled water.
2. The standard is a 5 mmol/L potassium dichromate solution, equivalent to 0.2 mg/mL pyrogallol red 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.

4. Standard Solution Dilution

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. In this step, directly measure the absorbance: add 0.2 mL standard solution to a micro glass cuvette or glass 96-well plate and measure absorbance at 430 nm. Record values 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. Assay Operation

Component	Assay Tube	Blank Tube
Air-dried soil sample (g)	0.02	0.02
Reagent I (μL)	120	--
Distilled water (μL)	--	120
Shake and mix thoroughly. 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 and measure absorbance at 430 nm.		

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

S-PPO Activity Calculation

1. Standard Curve Preparation

Use the standard tube concentration (X, μg/mL) and absorbance $\Delta A_{standard}$(Y, $\Delta A_{standard}$) 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 Calculation

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

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

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 can fall off easily, pre-rinse the pipette tip in the upper layer liquid 2-3 times before

pipetting, then transfer the measured volume.
3. Ether is volatile. After adding the upper solution to the cuvette, measure the absorbance 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 micro glass cuvette or glass 96-well plate is recommended.
6. Instruments and supplies to be prepared by the user: visible spectrophotometer or microplate reader, water bath or metal bath, adjustable pipette, micro glass cuvette or 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. Use the assay operation table above, or use the absorbance values from each standard well to prepare a standard curve with $R^2 \geq 0.99$, then obtain the calculation formula for sample analysis.

Visual Reference