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: Spectrophotometer

Catalog Number: BC0110

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	Liquid 20 mL×1	2-8℃
Reagent II	Powder×2	2-8℃
Standard	Liquid 10 mL×1	2-8℃

Solution Preparation:

1. Reagent II: Add 11 mL of distilled water to one Reagent II before use. Unused solution can be stored at 2-8℃ 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, desk centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, 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℃, then sieved by 30 -50 mesh sieve.

II. Determination procedure

1. Preheat spectrophotometer for more than 30 minutes, adjust the wavelength to 430 nm, set 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	1000	1000	0.1
2	0.1	1000	1000	0.05
3	0.05	1000	1000	0.025
4	0.025	1000	1000	0.0125
5	0.0125	1000	1000	0.00625
6	0.00625	1000	1000	0.003125
7	-	0	1000	0

Note: The following experiments require 1mL of standard for each standard tube.

4. Establishment of standard curve: Take 0 mg/mL standard product as blank tube, take 1 mL of diluted standard in the 1 mL glass cuvette and detect the absorbance at 430 nm. 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)

Reagent	Test Tube(T)	Control Tube(C)
Air-dried soil sample(g)	0.1	0.1
Reagent I (μL)	300	300
Reagent II (μL)	720	-
distilled water (μL)	-	720
Fully mixed, reaction at 30℃ for 1h, centrifugation at 5000g at 4℃ for 10min, 800μL of supernatant was taken into 1mL glass cuvette, absorption value A was determined at 430nm. Record as A_T, A_C. Calculate $\Delta A_T=A_T-A_C$.		

III. Calculation

According to the concentration (x, mg/mL) of the standard tube and the absorbance ΔAs (y, ΔAs), establish a standard curve. According to the standard curve, bring ΔA_T (y, ΔA_T) into the formula to calculate the sample concentration (x, mg/mL).

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.

S-PPO activity (U/g soil) = x × V_{ST} ÷ W÷T =24.48 × x ÷ W

T: Reaction time, 1 h=1/24 d;
V_{ST}: Extract solution volume, 1.02 mL;
W: Sample quality, g.

Note:

1. If the absorption value of the Test tube ΔA_T >1, the sample quality can be reduced for determination. If the absorption value of the Test tube ΔA_T<0.03, 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 0.103g Holly soil and follow the determination procedure. Using 1mL glass cuvette, measured $\Delta A_T=A_T-A_C$ =0.537-0.116=0.421. Bring it into the standard curve y=8.7342x+0.0149 and calculate x=0.046. Calculate S-PPO activity:
   S-PPO activity (U/g soil) = 24.48 × x ÷ W=10.933 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