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Soil Peroxidase(S-POD) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: AK0508

Size:50T/24S

Components:

Regent I: Powder×2, storage at 4℃ . Add 10 mL of distilled water when the solution will be used. It is suggested that the inexhaustible reagents should still be preserved at 4℃ .

Regent II: 5 mL×1, storage at 4℃ .

Regent III: 10 mL×1, storage at 4℃ .

Regent IV: Diethyl ether 100 mL×1, storage at 4℃ (self-provided reagent).

Standard: 10 mL×1, storage at 4℃ . Equivalent to 0.1 mg/mL of purple gallnut in per milliliter of diethyl ether.

Product Description：

S-POD is mainly derived from soil microorganisms, which can oxidize organic matter in soil to produce peroxide, which plays an important role in the process of humus formation. S-POD catalyze the oxidation of organic substances to quinones which has an absorption peak at 430 nm.

Reagents and Equipment Required but Not Provided：

Spectrophotometer, desk centrifuge, adjustable pipette, 1 mL glass cuvette, diethyl ether, mortar/homogenizer, ice and distilled water.

Procedure:

Ⅰ Sample preparation:

Fresh soil sample: Air-drying or drying at 37℃ oven, then passing through a 30-50 meshs sieve.

Ⅱ Determination procedure:

1. Preheat the spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 430 nm, set zero with Reagent Ⅳ,

2.Standards preparation: Dilute the standard with 0.5 mol/L HCl to 0. 1, 0.08, 0.06, 0.04, 0.02, 0.01, 0 mg/mL.

3. Establishment of standard curve: determine the absorbance of each concentration standard tube, and establish the standard curve according to the absorbance (x, minus the absorbance value of 0 concentration) and concentration (y).

4.

Reagent name (μL)	Test tube (A_T)	Substrate-free tube (A_S)
Air-dried soil sample(g)	0.05	0.05

Distilled water	-	100
Regent I (μL)	400	400
Regent II (μL)	100	-
Shake to mix thoroughly, incubate at 30℃ for 1 hour.		
Regent III (μL)	200	200
Regent IV (μL)	1750	1750
Mix thoroughly, place at room temperature for 30 minutes, set zero with distilled water. Take 1 mL of supernatant to detect the absorbance, record as A_T ,A_S respectively , $\Delta A=A_T - A_S$.		

Ⅲ Calculation:

According to the standard curve, bring ΔA(x) into the formula to calculate the y-value(mg/mL).

Unit definition: One unit of soil peroxidase activity is defined as the amount enzyme catalyzes the produce of 1 mg of purple gallnut every gram of soil sample per day.

S-POD (U /g soil sample) ＝y×V÷W÷T=840×y

V: The total volume of Extract solution, 1.75 mL;

T: Reaction time, 1 hour =1/24 day;

W: Sample weight, 0.05 g.

Note:

Each sample should provide one opposite substrate-free tube.

References：

[1] Doxey D L. The use of pyrogallol to demonstrate peroxidase in mammalian blood eosinophils[J]. Stain Technology, 1962, 37(6): 367-371.

[2] Nozaki O, Ji X, Kricka L J. New enhancers for the chemiluminescent peroxidase catalysed chemiluminescent oxidation of pyrogallol and purpurogallin[J]. Journal of bioluminescence and chemiluminescence, 1995, 10(3): 151- 156.

Related Products：

AK0594/AK0593 Soil Polyphenoloxidase Activity Assay Kit

AK0596/AK0595 Soil Catalase(S-CAT) Activity Assay Kit