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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/microplate reader

Catalog Number: AK0507

Size: 100T/48S

Components:

Regent I: Powder×1, storage at 4°C. 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°C.

Regent II: $2 \text{ mL} \times 1$, storage at 4°C .

Regent III: 5 mL×1, storage at 4°C.

Regent IV: Diethyl ether 50 mL×1, storage at 4°C (self-provided reagent).

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

Product Description:

S-POD mainly comes from soil microorganism, 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 quinone which has an absorption at 430 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, water-bath, adjustable pipette, micro glass cuvette/96-well flat-bottom plates, diethyl ether, mortar/homogenizer, ice and distilled water.

Procedure:

I Sample preparation:

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

I 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. Determination procedure:

| Reagent name | Test tube (A _T) | Substrate-free tube (A _S) |
|---------------------------|-----------------------------|---------------------------------------|
| Air-dried soil sample (g) | 0.02 | 0.02 |





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| Distilled water | - | 20 | |
|--|-----|-----|--|
| Regent I (µL) | 100 | 100 | |
| Regent II (μL) | 20 | _ | |
| Shake to mix thoroughly, culture at 30°C for 1 hour. | | | |
| Regent III (µL) | 50 | 50 | |
| Regent IV (µL) | 430 | 430 | |

Oscillation several times, stand at room temperature for 30 minutes, t zero with distilled water. Take 0.2 mL of supernatant to detect the absorbance, record as A_T , A_S respectively, calculate $\Delta A = A_T - A_S$.

Ⅲ Calculation:

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

S-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) = $y \times V \div W \div T = 516 \times y$

V: The total volume of Extract solution, 0.43 mL;

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

W: Sample weight, 0.02 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:

Soil Polyphenoloxidase Activity Assay Kit AK0594/AK0593

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