

Soil Lignin Peroxidase (S-Lip) Activity Assay Kit

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

Operation Equipment: Microplate reader/Spectrophotometer

Catalog Number: BC1975

Size: 100T/48S

Components:

Reagent I: 12 mL×1, stored at 4°C.

Reagent II: 15 mL×1, stored at 4°C.

Reagent III: 10 mL×1.

Product Description

Lignin peroxidase (EC1.11.1.14) is a kind of peroxidase containing heme, which belongs to lignin degradation enzyme system. It has great application potential in lignin biodegradation, papermaking industry, textile industry, aromatics transformation and degradation, and environmental pollution control. Resveratrol is oxidized by lignin peroxidase to form resveraldehyde, which has a characteristic absorption peak at 310 nm.

Reagents and Equipment Required but Not Provided.

Scales, low temperature centrifuge, ultraviolet spectrophotometer/microplate reader, micro quartz cuvette/96 well plate (UV), water bath, oscillating instrument, toluene (> 98%, AR), 30-50 mesh sieve (or smaller), distilled water.

Procedure

I. Sample processing:

The fresh soil samples are dried naturally and screened with 30-50 mesh.

II. Determination steps:

- Preheat ultraviolet spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 310 nm, ultraviolet spectrophotometer set zero with the distilled water.
- Add reagents in turn according to the following table:

| Reagent name | Test tube (T) | Contrast tube (C) |
|--|---------------|-------------------|
| Soil sample (g) | 0.04 | 0.04 |
| Toluene (μL) | 30 | 30 |
| Allow to stand for 15 minutes at room temperature. | | |
| Reagent I (μL) | 200 | - |
| H ₂ O | - | 200 |
| Reagent II (μL) | 120 | 120 |
| Reagent III (μL) | 80 | 80 |

After reaction in water bath at 30°C for 3 hour, Ice bath for 5 min. Then centrifuged at 12000 g 4 °C for 10 min, and 200 μL supernatant is carefully taken in a micro-quartz cuvette or a 96-well UV plate to determine the absorbance at 310 nm, which is recorded as A_T and A_C , respectively, and $\Delta A = A_T - A_C$.

Note: If the supernatant is not clear, all the supernatant can be taken out, and 200 μL supernatant can be taken after centrifugation.

III. Calculate activity of S-LiP

1. Calculated by micro glass cuvette

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of resveratrol in the reaction system per hour every gram soil sample.

$$\text{S-LiP (U/g weight)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div W \div T = 15.412 \times \Delta A \div W$$

ϵ : Molar extinction coefficient of resveratrol: 9300 L/mol/cm;

d : Light diameter of cuvette, 1 cm;

V_{RT} : The total volume of reaction, 430 μL = 4.3×10^{-4} L;

W : Mass of soil sample, g;

T : Reaction time, 3 hours;

10^9 : Unit conversion coefficient, 1mol = 10^9 nmol.

2. Calculated by 96 well plate(UV)

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the generation of 1 nmol of resveratrol in the reaction system per minute every g soil sample.

$$\text{S-LiP (U/g weight)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{RT} \div W \div T = 25.687 \times \Delta A \div W$$

ϵ : Molar extinction coefficient of resveratrol: 9300 L/mol/cm;

d : Light diameter of cuvette, 0.6 cm;

V_{RT} : The total volume of reaction, 430 μL = 4.3×10^{-4} L;

W : Mass of soil sample, g;

T : Reaction time, 3 hours;

10^9 : Unit conversion coefficient, 1mol = 10^9 nmol.

Experimental example:

- Take 2 pieces 0.04g Soil sample I to 1.5ml EP tube, one is test tube and the other is contract tube, operate as the procedure, $\Delta A = A_T - A_C = 1.069 - 0.374 = 0.695$, calculate content by sample weight:

$$\text{S-Lip Activity (U/g weight)} = 25.687 \times \Delta A \div W = 25.687 \times 0.695 \div 0.04 = 446.312 \text{ U/g weight.}$$

- Take 2 pieces 0.04g Soil sample II to 1.5ml EP tube, one is test tube and the other is contract tube, operate as the procedure, $\Delta A = A_T - A_C = 0.424 - 0.185 = 0.239$, calculate content by sample weight:

$$\text{S-Lip Activity (U/g weight)} = 25.687 \times \Delta A \div W = 25.687 \times 0.239 \div 0.04 = 153.480 \text{ U/g weight.}$$

Related products:

BC0110/BC0115 Soil Polyphenoloxidase Activity Assay Kit

BC0120/BC0125 Soil Urease(UE) Activity Assay Kit

BC0240/BC0245 Soil Saccharase(S-SC) Activity Assay Kit