Soil Leucine Aminopeptidase (S-LAP) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Catalog Number: BC4020

Size:50T/24S

Components:

Reagent I: 50 mL×1, stored at 4℃;

Reagent II: Powder×2. storage at 4℃. Before use, add 3 mL of acetone (self-provided reagent) into the bottle. The left reagent could be stored at 4℃ for one week.

Product Description

S-LAP is a kind of enzyme that can hydrolyzes the N-terminal of peptide chain to leucine, which is secreted by soil microorganism. The changes of S-LAP activity are closely related to some pathological states.

S-LAP decomposes L-leucine-p-nitroaniline to p-nitroaniline, the latter has the maximum absorption peak at 405 nm, and the activity of S-LAP is calculated by measuring the high rate of absorption value.

Reagents and Equipment Required but Not Provided.

Balance, desk centrifuge, water-bath, transferpettor, spectrophotometer, 1 mL glass cuvette, mortar, toluene, acetone, 30-50 mesh sieve, distilled water.

Procedure

I. Sample processing:

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

II. Determination steps:

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 405 nm, set zero with the distilled water.
2. Add reagents in turn according to the following table:

Reagent name	Test tube(T)	Contrast tube(C)
Soil sample (g)	0.1	0.1
Toluene (μL)	50	50
Shake and mix well, and let stand for 15 minutes at room temperature.		
Reagent I (μL)	850	850
Reagent II (μL)	100	-
After reaction in water bath at 30℃ for 1 hour, boil immediately for 5 minutes. Water cooling to room temperature.		

Reagent II (μL)	-	100

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Centrifugate at 14000×g for 10 minutes at room temperature, take supernatant and measure the absorbance value at 405 nm, record it as A_T and A_C respectively, calculate $\Delta A = A_T - A_C$.

III. Calculate activity of S-LAP

(1) Calculated by micro glass cuvette

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of p-nitrophenol per day every gram of soil sample.

S-LAP (U/g) = $\Delta A \div (\varepsilon \times d) \times 10^9 \times V_{RT} \div W \div T = 1.689 \times \Delta A \div W$

ε: Molar extinction coefficient of p-nitroaniline: 9.87×10^3 L/mol/cm;

d: Light diameter of cuvette, 1 cm;

V_{RT}: The total volume of reaction, 1 mL = 1×10^{-3} L;

W: Mass of soil sample, g;

T: Reaction time, 60 minutes;

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

Experimental Examples:

1. Take two tubes of 0.1g clover soil samples and record them as the measuring tube and the control tube respectively. Follow the measurement steps to calculate ΔA=At-Ac=0.982-0.223=0.759, and calculate the enzyme activity:
   S-LAP activity (U/g soil) ）=1.689×ΔA÷W=1.689×0.759÷0.1=12.8195 U/g soil.
2. Take two tubes of 0.1g soil sample and record them as the measuring tube and the control tube respectively. Follow the measurement steps to calculate ΔA=At-Ac=0.812-0.141=0.671, and calculate the enzyme activity:
   S-LAP activity (U/g soil) =1.689×ΔA÷W=1.689×0.671÷0.1=11.3332 U/g soil

Related Products:

BC0880/BC0885	Soil Alkaline Protease Activity Assay Kit
BC4010/BC4015	Soil β-Xylosidase(S-β-XYS) Activity Assay Kit
BC3080/BC3085	Soil α-glucosidase(S-α-GC) Activity Assay Kit
BC0240/BC0245	Soil Saccharase(S-SC) Activity Assay Kit

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