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Soil Leucine Aminopeptidase (S-LAP) Activity Assay Kit

Note: Take two or three different samples for prediction before test.
Operation Equipment: Microplate reader/Spectrophotometer

Catalog Number: AK0119
Size:100T/48S

Components:
Reagent I: 30 mL×1, stored at 4℃ .
Reagent II: Powder×1. storage at 4℃ and protected from light. Before use, add 3 mL of **acetone** (self-provided reagent) into the bottle, fully dissolve it.

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 405nm, and the activity of S-LAP is calculated by measuring the high rate of absorption value.

Reagents and Equipment Required but Not Provided.
Scales, centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, **toluene, acetone**, 30 mesh sieve (or smaller).

Procedure
I. Sample processing:
The fresh soil samples are dried naturally and screened with 30-50 mesh.
II. Determination steps:
1. Preheat spectrophotometer/microplate reader 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)	0.03	0.03
Toluene (μL)	15	15
Shake and mix well, and let stand for 15 minutes at room temperature.		
Reagent I (μL)	255	255
Reagent II (μL)	30	-
After reaction in water bath at 30℃ for 1 hour, boil immediately for 5 minutes. Water cooling to room temperature.		
Reagent II (μL)	-	30

Centrifugate at 14000 ×g for 10 minutes at room temperature, take 200 μL of 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) = $\Delta A \div (\varepsilon \times d) \times 10^9 \times V_{RT} \div W \div T = 0.507 \times \Delta A \div W$

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, 300 μL = 3×10^{-4} L;

W: Mass of soil sample, g;

T: Reaction time, 60 minutes;

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

(2) Calculated by 96 well plate

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) = $\Delta A \div (\varepsilon \times d) \times 10^9 \times V_{RT} \div W \div T = 0.844 \times \Delta A \div W$

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

d: Light diameter of cuvette, 0.6 cm;

V_{RT}: The total volume of reaction, 300 μL = 3×10^{-4} L;

W: Mass of soil sample, g;

T: Reaction time, 60 minutes;

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

Experimental Examples:

1. Take two tubes of 0.03g clover soil samples and record them as the measuring tube and the control tube respectively. Follow the measurement steps using 96-well plate to calculateto calculate ΔA=At-Ac==0.6-0. 17=0.43, and calculate the enzyme activity:
   S-LAP activity (U/g soil) ） =0.507×ΔA÷W=0.507×0.43÷0.03=7.267 U/g soil.
2. Take two tubes of 0.03g soil sample and record them as the measuring tube and the control tube respectively. Follow the measurement steps using 96-well plate to calculate ΔA=At-Ac=0.569-0. 128=0.441, and calculate the enzyme activity:
   S-LAP activity (U/g soil) =0.507×ΔA÷W=0.507×0.441÷0.03=7.4529 U/g U/g soil

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