Soil Lipase(S-LPS) Activity Assay Kit

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

Operation Equipment: Microplate Reader/Spectrophotometer

Cat No: NA0369

Size:100T/48S

Components:

Reagent I: 15 mL×1, storage at 4℃.

Reagent II: 5 mL×1, storage at room temperature.

Reagent III: Powder×1, storage at 4℃.

Reagent IV:10 mL×1, storage at 4℃.

Standard: 59.3 μL×1, storage at 4℃. Before use add 1.435 mL of toluene to obtain 125 μmol/ml oleic acid. Pay attention to thawing and dissolving before use.

Preparation of working solution: Add 16 mL of distilled water into the Reagent III in the boiling water bath to dissolve before use, cool it to room temperature, add 4 mL of Reagent II into the solution, mixing, shake it twice at high speed, 3 minutes of each time, 5 minutes of interval. Store at 4℃. Perpare when the solution will be used according to the proportion.

Product Description:

Lipase (LPS), also known as glyceride hydrolase, catalyzes the hydrolysis of triglycerides to produce fatty acids and glycerol (or diacylglycerol and monoesters). The enzyme plays an important role in soil biological dynamics.

LPS catalyzes the hydrolysis of oil esters to fatty acids. The activity of LPS can be calculated by measuring the rate of fatty acid formation with copper soap method.

Reagents and Equipment Required but Not Provided：

Desktop centrifuge, shaker mixer, spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate (non-polystyrene/polypropylene structure), transferpettor, toluene, ice and distilled water, 30 mesh sieve (or smaller).

Procedure:

I. Treatment of soil samples:

Natural air drying of fresh soil sample, passing 30-50 mesh sieve.

II. Determination steps:

1. Preheat Spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 710 nm, set zero with toluene.

2. Dilution of standard solution: dilute 125 μmol/mL oleic acid standard solution with toluene 25 times to 5 μmol/mL standard solution to be tested.

3. Add reagents as the following table.

Reagent name	Contrast tube(C)	Test tube(T)	Standard solution(S)	Blank tube(B)
Soil sample (g)	0.03	0.03	-	-
Toluene (μL)	15	15	-	-
The soil sample shall be fully wetted and placed at normal temperature for 10 minutes.			-	-
Reagent I (μL)	150	150	-	-
Working solution (μL)	-	150	-	-
During the reaction of water bath at 37℃ for 1 hours, the soil sample can be shaken several times to make full contact with the sample. After that, take a boiling bath for 10 minutes and cool it to room temperature.			-	-
Working solution (μL)	150	-	-	-
Toluene (μL)	360	360	-	-
After repeated shaking and mixing, centrifugation at 4000 rpm for 10 minutes at room temperature.			-	-

Take out the centrifuge tube, carefully suck 0.3 mL of the upper organic phase, add another 1.5 mL EP tube, and operate according to the following table：

the upper solution (μL)	300	300	-	-
standard solution (μL)	-	-	300	
Toluene (μL)	-	-	-	300
Reagent IV (μL)	75	75	75	75

After centrifugation at 4000 rpm for 10 minutes, carefully suck 200 μL of the organic phase solution, add it into the micro glass cuvette/96 well plate, and measure the absorption value at 710 nm. Record as A_C, A_T, A_S, A_B. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$.

III. LPS activity calculation:

Unit definition: One unit of enzyme activity is defined as that the amount of enzyme that catalyzes the hydrolysis of olive oil to generate 1 μmol fatty acid per day every gram soil sample at 37℃.

S-LPS (U/mg prot) $= \Delta A_T \div (\Delta A_S \div C_S) \times V_T \div T \div W = 43.2 \times \Delta A_T \div \Delta A_S \div W$

V_T: Volume of added toluene, 0.36 mL;

C_S: Concentration of standard solution, 5 μmol/mL;

T: Catalytic reaction time, 1/24d;

W: Fresh weight of sample, g.

Note:

1. Toluene is toxic. Gloves and masks should be worn during the experiment.
2. Keep away from fire during the experiment.

3. When the absorbance is greater than 0.8, it is recommended to dilute the sample for measurement (the amount of toluene added for the second time increases).
4. Toluene dissolves polystyrene/polypropylene.

Experimental examples:

1. Take two tubes of 0.03g clover soil and mark them as test tube and control tube respectively, and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T = A_T - A_C$ =0.145-0.093=0.052, $\Delta A_S = A_S - A_B$ =0.432-0.061=0.371. The enzyme activity is calculated according to the sample mass.
   S-LPS (U/g prot) =$43.2 \times \Delta A_T \div \Delta A_S \div W$ =200.33 U/g.
2. Take two tubes of 0.03g woodland and mark them as test tube and control tube respectively, and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T = A_T - A_C$ =0.145-0.093=0.052, $\Delta A_S = A_S - A_B$ =0.432-0.061=0.371. The enzyme activity is calculated according to the sample mass.
   S-LPS (U/g prot) =$43.2 \times \Delta A_T \div \Delta A_S \div W$ =170.78 U/g.

Related products:

NA0361/NA0360	Soil β-1,4-Glucanase Activity Assay Kit
NA0371/NA0362	Soil Leucine Arylamidase(S-LAP) Activity Assay Kit
NA0850/NA0608	Soil Saccharase(S-SC) Activity Assay Kit
NA0644/NA0402	Soil Nitrate Reductase(S-NR) Activity Assay Kit