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Soil Lipase (S-LPS) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0127

Size:50T/24S

Components:

Reagent I: 30 mL×1, storage at 4℃ .

Reagent II: 6 mL×1, storage at room temperature.

Reagent III: Powder×1, storage at 4℃ .

Reagent IV: 20 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 20 mL of distilled water into the Reagent III in the boiling water bath to dissolve before use, cool it to room temperature, add 5 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℃ . Prepare 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, 1 mL glass cuvette, 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. Take 2 mL EP tubes, add reagents as the following table.

Reagent name	Contrast tube(C)	Test tube(T)	Standard solution(S)	Blank tube(B)
Soil sample (g)	0.1	0.1	-	-
Toluene (μL)	50	50	-	-
The soil sample shall be fully wetted and placed at normal temperature for 10 minutes.			-	-
Reagent I (μL)	500	500	-	-
Working solution (μL)	-	500	-	-
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)	500	-	-	-
Toluene (mL)	1.2	1.2	-	-
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 (mL)	1	1	-	-
standard solution (mL)	-	-	1	
Toluene (mL)	-	-	-	1
Reagent IV(μL)	250	250	250	250

After centrifugation at 4000 rpm for 10 minutes, carefully suck 800 μL of the organic phase solution, add it into the 1 mL glass cuvette, 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/g prot) $= \Delta A_T \div (\Delta A_S \div C_S) \times V_T \div T \div W = 144 \times \Delta A_T \div \Delta A_S \div W$

V_T: Volume of added toluene, 1.2 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).

Experimental examples:

1. Take two tubes of 0. 1g clover soil and mark them as test tube and control tube respectively, and follow the measurement procedure. Calculate $\Delta A_T = A_T - A_C$ =0. 195-0.094=0. 101, $\Delta A_S = A_S - A_B$ =0.754-0.028=0.726. The enzyme activity is calculated according to the sample mass.
   S-LPS (U/g prot) = $144 \times \Delta A_T \div \Delta A_S \div W$ =200.33 U/g.
2. Take two tubes of 0. 1g woodland and mark them as test tube and control tube respectively, and follow the measurement procedure. Calculate $\Delta A_T = A_T - A_C$ =0. 143-0.074=0.069, $\Delta A_S = A_S - A_B$ =0.754-0.028=0.726. The enzyme activity is calculated according to the sample mass.
   S-LPS (U/g prot) = $144 \times \Delta A_T \div \Delta A_S \div W$ =136.86 U/g.

Related products:

AK0118/AK0117	Soil β- 1,4-Glucanase Activity Assay Kit
AK0120/AK0119	Soil Leucine Arylamidase(S-LAP) Activity Assay Kit
AK0574/AK0573	Soil Saccharase(S-SC) Activity Assay Kit
AK0370/AK0369	Soil Nitrate Reductase(S-NR) Activity Assay Kit