CheKine™ Mirco Soil Lipase (S-LPS) Activity Assay Kit

Cat #: KTB4026 Size: 48 T/48 S 96 T/96 S

	Mirco Soil Lipase (LPS) Activity Assay Kit		
REF	**Cat #:** KTB4026	LOT	**Lot #:** Refer to product label
	Applicable sample: Soil		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Lipase (LPS), also known as glycerol ester hydrolase, catalyzes the hydrolysis of triglycerides to produce free fatty acids and glycerol (or di- and mono-glycerides). LPS is widely distributed in various organisms. CheKine™ Mirco Soil Lipase (S-LPS) Activity Assay Kit offers a simple, convenient, and rapid approach for assessing soil LPS activity. It works by having LPS catalyze the hydrolysis of oil esters into fatty acids, and by measuring the rate of fatty acid production using the copper soap method, the LPS activity can be calculated.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	80 mL	80 mL×2	4℃
Reagent Ⅱ	6 mL	12 mL	4℃, protected from light
Reagent Ⅲ	12 mL	24 mL	4℃, protected from light
Standard	10 μL	10 μL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 710 nm
- Incubator, orbital shaker, ice maker, freezing centrifuge, 30-50 mesh sieve
- 96-well quartz/glass plate (non-polystyrene material) or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water, toluene
- Dounce homogenizer

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Before use, equilibrate to room temperature and vortex vigorously for 20 min using a vortex mixer before each use. Store at 4℃, protected from light.

ReagentⅢ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Standard: Prepared before use. Add 3.168 mL of toluene and dissolve completely to obtain a 10 μmol/mL oleic acid standard solution. Unused 10 μmol/mL standard can be stored at 4℃ for up to one month.

Sample Preparation

Note: It is recommended to use fresh soil samples.

Fresh soil samples naturally air dried or air dried in an oven at 37℃ and sieved through 30-50 mesh sieve. Weigh 0.1 g Soil, add 1 mL Reagent Ⅰ and homogenize on ice. Then shake and extract in thermostatic shaker at 37℃ for 15 min. Centrifuge at 4,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 710 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent Ⅰ and Reagent Ⅱ in a 37℃ water bath for more than 30 min.
3. Operation table (The following operations are operated in a 1.5 mL EP tube):

Reagent	Blank Tube (μL)	Test Tube (μL)	Standard Tube (μL)
Deionized Water	150	0	0
Sample	0	50	0
Reagent Ⅰ	300	300	0
Reagent Ⅱ	0	100	0
Incubate and agitate at 37℃ for 20 min			
Toluene	800	800	0
After oscillating and reacting at 37℃ for 10 min, centrifuge at 8,000 g and 25℃ for 10 min, then collect the supernatant			
Supernatant	400	400	0
10 μmol/mL Standard	0	0	400
ReagentⅢ	100	100	100
After oscillating and reacting at 37℃ for 5 min, Centrifuge at 12,000 g for 10 min at room temperature. Take 200 μL of the upper layer liquid and transfer it to a micro glass cuvette or a 96-well quartz/glass plate (non-polystyrene material). Measure the absorbance at 710 nm, recording the values as A_{Blank}, A_{Test} and $A_{Standard}$. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.			

Note: Standard Tube and Blank Tube only need to be tested 1-2 times. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.01, it is advisable to increase the sample volume appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Reagent Ⅰ, or the sample extracted with toluene (800 μL) in the first step can be further diluted with toluene, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly

equal. It is suggested that the concise calculation formula in bold is final formula.

Active unit definition: one enzyme activity unit is defined as the amount of enzyme that hydrolyzes olive oil to produce 1 μmol of fatty acids per min, per g of soil, at a temperature of 37℃.

S-LPS (U/g Soil)=($C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$)×V_{Total}÷(W×V_{Sample}÷$V_{Total\ Sample}$)÷T=**8×ΔA_{Test}÷$\Delta A_{Standard}$÷W**

Where: $C_{Standard}$: Standard concentration, 10 μmol/mL; V_{Total}: Extraction volume of toluene, 0.8 mL; V_{Sample}: Volume of sample added in the reaction, 0.05 mL; $V_{Total\ Sample}$: Volume of Reagent Ⅰ added, 1 mL; W: Sample mass, g; T: Catalytic reaction time, 20 min.

Precautions

1. Toluene is toxic; gloves and a mask should be worn during the experiment, and it is recommended to operate in a fume hood.
2. During the experiment, keep away from any source of fire.

Typical Data

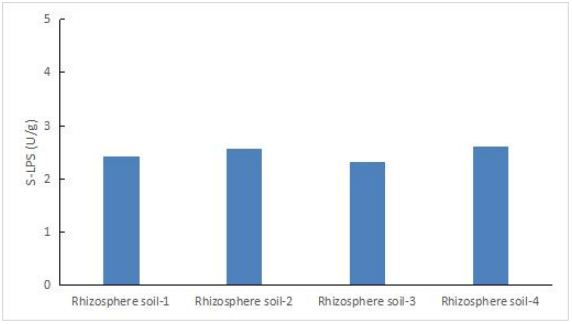

Figure 1. Determination LPS activity in rhizosphere soil samples by this assay kit

Recommended Products

Catalog No.	Product Name
KTB4018	CheKine™ Mirco Soil Urease (S-UE) Activity Assay Kit
KTB4023	CheKine™ Mirco Soil Peroxidase (S-POD) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.