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CheKine™ Mirco Lipase (LPS) Activity Assay Kit

Cat #: KTB2241 Size: 48 T/48 S 96 T/96 S

	Mirco Lipase (LPS) Activity Assay Kit		
REF	**Cat #:** KTB2241	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells, Serum (Plasma)		
	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. Elevated levels of LPS in the serum are commonly observed in pancreatitis and pancreatic cancer. CheKine™ Mirco Lipase (LPS) Activity Assay Kit provides a simple, convenient, and rapid method for detecting LPS activity, applicable to samples such as animal and plant tissues, cells, serum, and plasma. 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	80×2 mL	4℃
Reagent Ⅱ	6 mL	12 mL	4℃, protected from light
Reagent Ⅲ	6 mL	12 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
- 96-well plate (non-polystyrene material) or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, orbital shaker, ice maker, freezing centrifuge
- Deionized water, toluene
- Dounce homogenizer (for tissue samples)

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. Store at 4℃, protected from light.

Reagent III: 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 reagent can be stored at 4℃ for up to one month.

Note: Reagent II, Reagent III and Standard have a pungent odor, so it is recommended to experiment in a fume hood.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Animal and tissues: Weigh 0.1 g tissue, add 1 mL Reagent I and homogenize on ice. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 10^7 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent I to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Test directly.

Note: 1. After centrifuging high-fat samples, if there is solid lipid present above the supernatant, it must be wiped away with a cotton swab or similar before proceeding with the measurement.

2. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

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 10 min			
Toluene	800	800	800
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
Standard	0	0	400
Reagent III	100	100	100
After oscillating and reacting at 37℃ for 5 min, allow it to stand for an additional 5 min. Take 200 μL of the upper layer liquid and transfer it to a micro glass cuvette or a 96-well 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: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA is less than 0.01, it is advisable to increase the sample volume appropriately. If ΔA is greater than 1.0, the sample can be appropriately diluted with Reagent I, 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.

1. Calculated by protein concentration

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 mg of protein, at a temperature of 37℃.

LPS (U/mg prot)=$(C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard})\times V_{Total}\div(Cpr\times V_{Sample})\div T$**$=16\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr$**

2. Calculated by sample fresh weight

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 tissue, at a temperature of 37℃.

LPS (U/g fresh weight)=$(C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard})\times V_{Total}\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**$=16\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

3. Calculated by cell number

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 10^4 of cells, at a temperature of 37℃.

LPS ($U/10^4$)=$(C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard})\times V_{Total}\div(N\times V_{Sample}\div V_{Total\ Sample})\div T$**$=16\times\Delta A_{Test}\div\Delta A_{Standard}\div N$**

4. Calculated by liquid volume

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 mL of sample, at a temperature of 37℃.

LPS (U/mL)=$(C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard})\times V_{Total}\div V_{Sample}\div T$**$=16\times\Delta A_{Test}\div\Delta A_{Standard}$**

Where: $C_{Standard}$: Standard concentration, 10 μmol/mL; V_{Total}: Total reaction volume, 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; Cpr: Supernatant protein concentration, mg/mL; W: Sample mass, g; N: Total number of cells, 10^4; T: Catalytic reaction time, 10 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

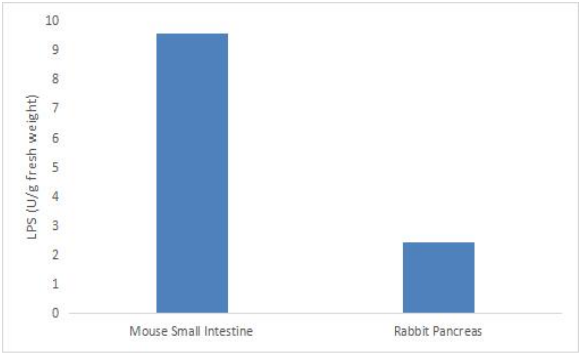

Figure 1. Determination LPS activity in Mouse Small Intestine and Rabbit Pancreas by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric 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.