



Lipase (LPS) Assay Kit

(Cat/No.: BC055 Size:50T/48S)

1. Principle of Measurement

The latex prepared from triglycerides and water exhibits a turbid nature due to the absorption and scattering of incident light by its micelles. Under the action of Lipase (LPS), the triglycerides in the micelles are hydrolyzed, leading to the disruption of the micelles. Consequently, the scattered light or turbidity decreases, and the rate of this decrease is related to the lipase activity.

2. Composition and Preparation: (The kit is valid for 6 months)

Reagent 1: Substrate Buffer Solution, 60 mL per 2 bottles, stored at 4°C, and used after pre-warming at 37°C before use.

Reagent 2: Tris buffer solution, 10 mL per bottle, stored at 4°C;

Reagent 3: Normal Saline, 10 mL per bottle. It should be stored at 4°C for short-term use and at -20°C for long-term storage.

Reagent 4: 10 mL per bottle, stored under refrigeration (4°C).

All reagents should be left at room temperature for half an hour before the test.

3. Required Instruments and Reagents

Visible light spectrophotometer, 1 cm cuvette, vortex mixer, 37°C water bath, protein determination reagent (for tissue samples, available from our company).

4. Measurement of serum (plasma) lipase

1. Operation process:

- ①、Set the spectrophotometer at 420nm, with a 1 cm glass cuvette, and zero it using Tris buffer solution.
- ②、Preheat the substrate buffer at 37°C for more than 5 minutes.
- ③、Add 50 μ L of serum (plasma) to the corresponding numbered test tubes, then add 2 mL of the pre-warmed substrate buffer solution to the tubes, mix quickly and start timing.
- ④、Immediately pour it into the cuvette, and measure the absorbance at 420nm using a spectrophotometer. Read the absorbance value A1 at 30 seconds.
- ⑤、Pour this colorimetric solution into the original test tube and place it in an accurate 37°C water bath for 10 minutes. Then, quickly transfer it to the colorimeter and read the absorbance value A2 at 10 minutes and 30 seconds.
- ⑥、Calculate the difference in absorbance values for two measurements ($\Delta A=A1-A2$).

2、Calculation formula:

Definition of serum (plasma) lipase units: At a temperature of 37°C, each liter of serum (plasma) was reacted with the substrate in this reaction system for 1 minute. One enzyme activity unit was defined as the consumption of 1 μ mol of the substrate.

Calculation of the absorbance of the standard tube : Take 2 mL of the substrate buffer solution and add 50 μ L of physiological saline. Measure the turbidity at 420 nm, and read



the absorbance value (AS value). This AS value is equivalent to the absorbance value of the standard tube (454 μmol/L) at the same concentration.

Serum (plasma) lipopolysaccharide (LPS) activity (unit: U/L)=

$$\frac{A_1 - A_2}{A_s} \times C_{standard} \times \frac{V_{total\ reaction}}{V_{sample}} \div T$$

C_{standard}: Standard concentration, 454 μmol/L;

V_{total reaction}: Total volume of the reaction solution is 2.05 mL.

V_{sample}: Sample volume, 0.05 mL

T: Reaction time, 10 minutes.

3、 Notes:

- ① 、 Before each colorimetric test, the cuvette should be thoroughly washed with warm distilled water and then used for the next test.
- ② 、 Approximately 3 to 5 percent of the analyzed specimens show an increase in absorbance after interacting with the substrate. This usually occurs in specimens that have been repeatedly frozen and thawed, as well as in samples with elevated IgM levels (such as rheumatoid factor).

4、 For example:

Take 0.05 mL of porcine serum and perform the test according to the operation procedure. The measured *A_s* value is 0.729, *A₁* value is 0.916, and *A₂* value is 0.891. Then, the LPS activity of this porcine serum is:

$$\text{Porcine serum activity (U/L)} = \frac{0.916 - 0.891}{0.729} \times 454 \times \frac{2.05}{0.05} \div 10 = 63.834 \text{ U/L}$$

5. Determination of tissue lipase

1、 Operation process:

- ① 、 Set the spectrophotometer at 420nm, with a 1 cm glass cuvette, and zero it using Tris buffer solution.
- ② 、 Pre-warm the substrate buffer at 37°C for more than 5 minutes;
- ③ 、 Add 25 μL of **20% tissue homogenate supernatant** to the corresponding numbered test tube, then add 25 μL of reagent four. Draw 2 mL of pre-warmed substrate buffer and pour it into the test tube, mix quickly and start timing.
- ④ 、 Rapidly pour it into the cuvette and measure the turbidity at 420 nm on the spectrophotometer. Read the absorbance value *A₁* at 30 seconds.
- ⑤ 、 Pour this colorimetric solution into the original test tube and place it in a precisely controlled water bath at 37°C for 10 minutes. Then, quickly transfer it into the colorimetric cell and read the absorbance value *A₂* at 10 minutes and 30 seconds.
- ⑥ 、 Calculate the difference in absorbance values for two measurements (Δ*A*=*A₁*-*A₂*) .

2、 Calculation formula:

Definition of Tissue Lipase Uni : At a temperature of 37°C, each gram of tissue protein reacts with the substrate in this reaction system for 1 minute. One enzyme activity unit is defined as the consumption of 1 μmol of the substrate.

Calculation of the absorbance of the standard tube : Take 2 mL of the substrate buffer



solution and add 50 μL of physiological saline. Measure the turbidity at 420 nm, and read the absorbance value (AS value). This AS value is equivalent to the absorbance value of the standard tube (454 $\mu\text{mol/L}$) at the same concentration.

$$\text{Tissue LPS activity (U/gprot)} = \frac{A_1 - A_2}{A_s} \times C_{\text{standard}} \times \frac{V_{\text{total reaction}}}{V_{\text{sample}}} \div T \div C_{\text{pr}}$$

C_{standard} : Standard concentration, 454 $\mu\text{mol/L}$;

$V_{\text{total reaction}}$: Total volume of the reaction solution is 2.05 mL.

V_{sample} : Sample volume, 0.05 mL

T: Reaction time, 10 minutes.

C_{pr} : Tissue homogenate protein concentration, gprot/L (where "prot" stands for protein)

3、 Notes:

- ①、 **Preparation of 20% tissue homogenate:** Accurately weigh the wet weight of the tissue. According to the ratio of weight (g) : volume (mL) = 1 : 4, add 4 times the volume of normal saline. Perform mechanical homogenization under an ice-water bath condition. Centrifuge at 2500 revolutions per minute for 10 minutes. Take the supernatant for measurement.
- ②、 The content of lipase in the pancreas is very high. Before conducting the measurement, a preliminary experiment needs to be carried out to determine the optimal sampling concentration. ($\Delta A \leq 0.4$).
- ③、 Before each colorimetric test, the cuvette should be thoroughly washed with warm distilled water and then used for the next test.
- ④、 Approximately 3 to 5 percent of the analyzed specimens show an increase in absorbance after interacting with the substrate. This usually occurs in specimens that have been repeatedly frozen and thawed, as well as in samples with elevated IgM levels (such as rheumatoid factor).

4、 For example:

Take 0.025 mL of 20% liver and pancreas homogenate and conduct the measurement as per the operation. The results show that A_s is 0.716, A_1 is 1.068, and A_2 is 0.989. At the same time, the protein concentration of this 20% liver and pancreas homogenate solution is 15.346 gprot/L. Then, the LPS activity of this liver and pancreas tissue is:

$$\text{Tissue LPS activity (U/gprot)} = \frac{1.068 - 0.989}{0.716} \times 454 \times \frac{2.05}{0.025} \div 10 \div 15.346 = 26.732 \text{ U/L}$$

6. Detection scope

5.0-2000U/L