GENCHEM® Lipase Assay Kit

REF : KBCA1721

Ver 1.0

RUO

Quantitative Determination of Lipase in serum or plasma and other biological samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBCA1721 Σ 50 tests

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Introduction:

The GENCHEM® Assay kits are used for assessing the specific biomarker in samples analytes which may be serum or plasma as validated with the kit. The kit employs in vitro quantitative determination of Lipase in serum or plasma sample.

Intended Use:

This kit can be used to measure Lipase activity in tissues, serum (plasma), culture cells, cell culture supernatants and other samples.

Principle:

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.

Materials Provided:

1. Reagent 1 (Substrate Buffer Solution) – 60 ml x 2 vials
2. Reagent 2 (Tris Buffer Solution) – 10 ml
3. Reagent 3 (Normal Saline) – 10 ml

Handling/Storage:

This kit can be stored at 2~8°C away from the light for 1 year. Keep away from frozen and keep away from heat in transit. Once the reagents are opened, the reagent can be kept at 2~8°C and used within 1 month.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.



Sample Preparation:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1.Serum/plasma: Centrifuge before use. Avoid hemolysis. Enzyme activity keeps stable within 7 days at 2°C - 8°C, and keeps stable within 3 months at -20°C.
2.Tissue samples: Accurately weigh tissue weight, add normal saline according to the proportion of weight (g): volume (ml) = 1: 9. Use a mechanical homogenizer under ice water bath to prepare a 10% tissue homogenate, then centrifuge at 2500 RPM for 10 minutes, and extract the supernatant.
3. Cells sample: Homogenize the cells sample with homogenizer on ice, then extract the supernatant for detection.

Reagent Preparation

Reagent 1: Substrate Buffer Solution is ready to use, stored at 4°C, and used after pre-warming at 37°C before use.
Reagent 2: Tris buffer solution is ready to use, stored at 4°C.
Reagent 3: Normal Saline is ready to use, should be stored at 4°C for short-term use and at -20°C for long-term storage.

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

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).

Measurement of serum (plasma) lipase:

Assay Procedure:

1. Set the spectrophotometer at 420 nm, 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 ul 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（ΔA=A1-A2).

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 umol of the substrate.

Calculation of the absorbance of the standard tube : Take 2 ml of the substrate buffer solution and add 50 ul 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 umol/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 umol/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.

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).

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

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

Determination of tissue lipase:

Assay Procedure:

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 ul of **20% tissue homogenate supernatant** to the corresponding numbered test tube, then add 25 ul of reagent four. Draw 2 mL of pre-warmed substrate buffer and pour it into the test tube, 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 a precisely controlled water bath at 37°C for 10 minutes. Then, quickly transfer it to 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（△A=A1-A2).

Results Interpretation:

Definition of Tissue Lipase Unit: 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 umol of the substrate.

Calculation of the absorbance of the standard tube: Take 2 ml of the substrate buffer solution and add 50 ul 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 umol/l) at the same concentration.

Tissue LPS Activity (U/gprot) =

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

$C_{standard}$: Standard concentration, 454 umol/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.
Cpr : Tissue homogenate protein concentration, gprot/l (where "prot" stands for protein)

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 (△A≤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).

For Example:
Take 0.025 mL of 20% liver and pancreas homogenate and conduct the measurement as per the operation. The results show that As is 0.716, A1 is 1.068, and A2 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:

Tissue LPS activity (U/gprot) =

$$\frac{1.068 - 0.989}{0.716} \times 454 \times \frac{2.05}{0.025} \div 10 \div 15.346 = 26.732\ U/l$$

Detection Range: 5.0-2000 U/l

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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