



Ver.251201

Lipase (LPS) Assay kit

BC9903-01 (60 Tests)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Principle

In the presence of colipase and bile acids lipase splits the synthetic substrate (1,2- O-Dilauryl-rac-glycero-3-glutaricacid (6-methyl-resorufin-ester) to glycerol and methylresorufin-ester, which is spontaneously degraded to glutaric acid and methylresorufin. The rate of methylresorufin formation, measured photometrically is proportional to the catalytic concentration of lipase present in the sample. Wavelength measured at 580 nm.

Sensitivity

Lower detection limit is 3 U/L

Kit components

Reagent / Component	Volume	Storage
Lipase (S.L) R1	6 × 10mL	2-8°C
Lipase (S.L) R2	3 × 5mL	2-8°C
Lipase Calibrator	3 × 3mL	2-8°C

Reagent Preparation

- Reagent 1 & 2 are ready to use.
- Calibrator: Reconstitute with 3 mL of distilled water. Dissolve the content of the vial by swirling gently to avoid the formation of foam. Reconstituted calibrator is stable only for 7 days at 2-8°C and 3 months at -20°C.

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat bottom plate and distilled water.

Reagent Storage and Stability

The sealed reagents are stable up to the expiry date stated on the label, when stored at 2- 8°C and protected from light. Reconstituted calibrator is stable only for 7 days at 2-8°C or 3 months at -20 °C.

Open Vial Stability

Once opened, the reagent is stable up to 4 weeks at 2-8°C if contamination is avoided.

Reagent Deterioration

Turbidity or precipitation in any kit component indicates deterioration and the component must be discarded.

Precaution

- To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.
- Avoid direct exposure of reagent to light. Do not blow into the reagent bottles.

Sample Preparation

1. Bacteria or cells

Harvest the cells and wash twice with PBS. Ideal to use 5 million cells for the assay, Add 1mL. Extraction Reagent to 5 million cells and ultrasonicate (200W, work time 3 second/interval 10 second repeat for 30 times) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant. The supernatant should be kept on ice.

Note: Ideal proportion of Cells/Bacteria to Extraction Reagent is 1:5-10.

2. Tissue

Prepare 10% tissue homogenate by adding 1mL Extraction Reagent to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant

3. Serum or Plasma

Directly use for the assay.

Unit Conversion

Traditional Unit	SI Unit	Conversion from Traditional to SI
U/L	uKat/L	$\times 0.017$

Procedure

	Blank	Calibrator	Sample
Reagent 1	1000 μ L	1000 μ L	1000 μ L
Calibrator	-	20 μ L	-
Sample	-	-	20 μ L
Distilled water	20 μ L	-	-
Mix carefully (do not vortex); incubate for 1-5 minutes at 37°C. Then add			
Reagent 2	250 μ L	250 μ L	250 μ L
Mix and incubate for 2 minutes at 37°C, read absorbance against reagent blank. Measure the change in absorbance per minute (Δ OD /minutes) during 2 minutes.			
or			
Mix and read the optical density (T_1) 120 seconds after the Reagent 2 addition. Take second reading (T_2) exactly after 120 seconds.			

Calculation

$$\text{Lipase U/L} = \frac{(\Delta \text{ OD/minutes}) \text{ Sample} - (\Delta \text{ OD/minutes}) \text{ Blank}}{(\Delta \text{ OD/minutes}) \text{ Calibrator} - (\Delta \text{ OD/minutes}) \text{ Blank}} \times 113$$

$$(\Delta \text{ OD/minutes}) \text{ Calibrator} - (\Delta \text{ OD/minutes}) \text{ Blank}$$

or

$$\frac{(T_2 - T_1) \text{ of sample}}{(T_2 - T_1) \text{ of calibrator}} \times 113$$

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