

Manual Procedure

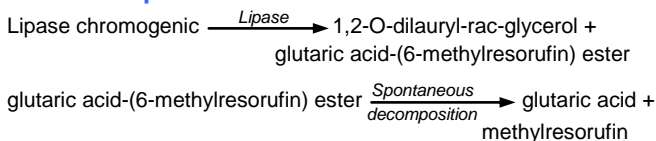
Cat. No. 12771 R 1 1 x 10 ml
For 10 tests R 2 1 x 2 ml

Lipase

Colorimetric method

Liquid Reagents

Test Principle



Lipase chromogenic: 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester

The chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester is cleaved by the catalytic action of alkaline lipase solution to form 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6-methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. The color intensity of the red dye formed is directly proportional to the lipase activity and can be determined photometrically.

Concentrations in the test

Reagent R1:			
Tris buffer pH = 8.3	40	mmol/L	
Co-Lipase	≥ 40	U/L	
Detergent, preservative			
Reagent R2: Substrate			
Buffer pH = 4.0			
Chromogenic substrate	0.7	mmol/L	
Detergent, preservative			
Calibrator : The concentration as indicated on vial.			

Stability of reagent

Reagent R1: liquid, ready to use.

Reagent R2: liquid, ready to use.

All reagents are stable up to expiry date given on label when stored at 2 - 8 °C.

Stability : 90 days at 2 - 8 °C after opened, if contamination avoided and vial recapped immediately after use .

Calibrator

Lipase Cal. Cat. No. 16271

Lipase Calibrator is a lyophilized calibrator.

Calibrator Preparation: Gently remove the stopper avoiding lost of lyophilized and add exactly 1.0 mL of distilled water. Replace stopper and gently swirl.

Stability : Lipase Calibrator is stored at 2 – 8 °C in unopened vial, is stable up to expiry date indicated on the vial .

The components of the reconstituted calibrator are stable for :

7 days at 2 – 8 °C, if contamination avoided and vial recapped immediately after use or 3 months at - 20 °C if aliquoted in small volumes. Each aliquote should be thawed only once .

Specimen collection and handling

1. Fasting, non-hemolyzed serum is the specimen of choice.
2. Serum should be separated immediately after collection and the lipase activity assayed promptly. If the assay is not performed immediately, the serum must be refrigerated or frozen until use. Never repeat freeze and thaw as the lipase can be inactivated.
3. The sample can be stored for 3 days at 2 - 8 °C.

Calibrator

MediCal U Cat. No. 15011

Quality control

Meditrol N Cat. No. 15171

Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 578 (570 - 590nm)
Spectrophotometer	580 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against distilled water
Reaction	kinetic – increase

Assay: Incubate reagents at 37 °C before use:

	Blank	Calibrator	Sample
Distilled water	10 µl	--	--
Calibrator	--	10 µl	--
Sample	--	--	10 µl
Reagent R1	1000 µl	1000 µl	1000 µl
Reagent R2	200 µl	200 µl	200 µl

Mix, incubate for 1 min. at 37 °C. Read change in absorbance against distilled water per 1 min. for 2 min. Determine the mean absorbance change per 1 min. ($\Delta A/\text{min}$).

Calculate the ($\Delta A/\text{min}$.) found for the Blank, Calibrator and Sample.

Calculation

$$\text{Conc. Lipase (U/L)} = \frac{\Delta A/\text{min. Sample} - \Delta A/\text{min. Blank}}{\Delta A/\text{min. Calibrator} - \Delta A/\text{min. Blank}} \times \text{Conc. Calibrator (U/L)}$$

Linearity

Up to 125 U/L.

If result exceeds 125 U/L, repeat test using sample diluted (1+9) with sodium chloride solution (0.9 %) and multiply result by 10.

Interferences

1. Icterus: No significant interference of bilirubin in a concentration up to 20 mg/dl.
2. Hemolysis: No significant interference of hemoglobin in a concentration up to 150 mg/dl.
3. Lipemia: No significant interference of triglycerides in a concentration up to 2000 mg/dl.
4. Drugs and other substances which may affect serum lipase level have been listed by Young.³

Precautions

Don't pipette the reagents by mouth.

Reagent R2 is turbid orange-colored micro-emulsion,
Discard if turning to red.

Reference range

Lipase in normal range ≤ 38 U/L.

References

1. Kazmierczak S, Catrou P, Van Lente F. Diagnostic accuracy of pancreatic enzymes evaluated by use of multivariate data analysis. *Clin Chem* 1993;39:1960-1965.
2. Panteghini M et al. Diagnostic value of four assays for lipase determination in serum: A comparative reevaluation. *Clin Biochem* 1991;24:497-503.
3. Neumann U et al. New substrates for the optical determination of lipase. *EP* 207252 (1987).
4. Tietz NW. *Clinical Guide to Laboratory Tests*, 3rd ed. Philadelphia, PA: WB Saunders, 1995:865.
5. Young, DS., *Effects of Drugs on Clinical Laboratory Tests*, fifth edition 2000, AACC Press, Washington, D.C.
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