LIPASE

(Colorimetric method)
Liquid Reagent

INTENDED USE:

This reagent kit is used for *in-vitro* quantitative determination of Lipase in human serum/plasma.

INTRODUCTION:

Lipase is a digestive enzyme released into the intestine from the pancreas where it breaks down triglycerides into fatty acids and glycerol prior to absorption. Lipase measurements are used in the diagnosis and treatment of diseases of the pancreas such as acute pancreatitis, obstruction of the pancreatic duct and pancreatic tumours.

TEST PRINCIPLE:

The colorimetric method is based on a lipase specific degradation of a chromogenic substrate. The specific lipase substrate-DGGMR [1,2-o-dilauryl-racglycero-3-glutaric acid-(6'-methylresorufin) ester] is cleaved by the catalytic action of lipase to form 1,2-odilaurylracglycerol and an unstable intermediate, glutaric acid-(6- methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. The lipase activity in the specimen is proportional to the production of methylresorufin in the reaction and can be determined photometrically.

KIT CONTENTS:

Reagent 1: R1 Lipase Reagent.

Reagent 2: R2 Lipase Reagent.

Reagent 3: Calibrator.

Product Insert

The calibrator value is mentioned on the vial label.

WORKING REAGENT PREPARATION:

Assay can be performed with use of separate R1 and R2 or with use of working reagent. For Working reagent preparation mix gently 5 parts of R1 and 1 part of R2 reagent. Since R2 is coloured in nature, do not store working reagent for prolonged usage. Always use freshly prepared working reagent for better absorbance.

REAGENT STABILITY AND STORAGE:

All the reagents must be stored at 2-8^{o}C and are stable till expiry date mentioned on the labels.

PRECAUTIONS: ⚠

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assy bring all the reagents to room temperature.
4. Avoid contamination of the reagent during assay process.
5. Use clean glassware free from dust or debris.
6. Reagent ratio as mentioned here above must be strictly observed as may change into it will adversly effect the factor.

SPECIMEN COLLECTION AND STORAGE:

Serum, heparinized plasma free from hemolysis. Sample may be stored for up to 5 days at 2-8°C or 24 hours at 20-25°C. Nevertheless it is recommended to perform the assay with freshly collected samples. Serum, heparinized or EDTA plasma.

PROCEDURE (Automated):

Refer to specific instrument application instructions.

TEST PROCEDURE (Manual):

Wevelength: 578 nm & Temperature: 37°C

Note : Bring reagents and samples to room temperature (21-25°C).

PIPETTE INTO THE TUBE:

Reagent	Calibrator(C)	Test (T)
R1 Lipase Reagent	1000 µl	1000 µl
R2 Lipase Reagent	200 µl	200 µl
Mix well and bring to assay temperature, then add		
R3 Calibrator	10 µl	
Sample		10 µl

Mix well and after exactly 60 secs read the absorbance A1 of the Test (T) and Calibrator (C) against air or water. In next 60 secs repeat absorbancereading A2 and calculate ΔA (A2-A1) for test and calibrator

CALCULATION:

Lipase activity [U/L] = ΔA (T) / ΔA (C) x Calibrator concentration

NORMAL VALUES*:

5 -60 U/L

*It is recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS:

1. **Sensitivity / Limit of Quantitation: 5 U/L.**
2. **Linearity:** up to 250 U/L. If the sample activity exceeds 250 U/L, dilute sample with 0.9% NaCl and repeat the assay. Multiply the result by the dilution factor.
3. **Specificity / Interferences:**
   Haemoglobin up to 2.5 g/dl, bilirubin up to 20 mg/dl, triglycerides up to 500 mg/dl, ascorbate up to 62 mg/l do not interfere with the test.

SYSTEM PARAMETERS:

Method	Fixed Time (2-point)
Wavelength	578 nm
Zero Setting Temperature	Distilled Water
Setting Incubation	37° C
Temperature Incubation	37° C
Time	----
Delay Time	60 secs
Read Time	60 secs
No. of Reading	2
Interval Time	----
Sample Volume Reagent	0.01 ml (10 ul)
Volume	1.2 ml (1200 ul)
Calibrator Concentration	Refer Calibrator vial
Units	U/L
Factor	----
Reaction Slope	Increasing
Linearity	250 U/L

QUALITY CONTROL:

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls

REFERENCE:

1. Tietz NW et al. Lipase in serum-the elusive enzyme: An overview. Clin Chem 1993;39:746-756.
2. Steinberg WM, Goldstein SS,Davies ND et al. Diagnostic assays in acute pancreatitis. (Review). Ann Intern Med 1985; 102:576-580.
3. Leybold A, Junge W. Importance of colipase for the measurement of serum

Manufactured by:
Vanguard Diagnostics Private Limited
C-123, Phase-I, Okhla Industrial Area
New Delhi-110 020, INDIA.

Tel: 011-4172 7222,
Email:info@vanguarddiagnostics.com
Website : www.vanguarddiagnostics.com

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