LIPASE KIT

(Turbidimetric U.V.Method)

For the determination of Lipase activity in Serum.
(For In vitro diagnostic use only)

Summary:

Lipases are enzymes which hydrolyse glycerol esters of long chain fatty acids. The enzyme and its cofactor, colipase is produced in the pancreas, lipase being also secreted in small amounts by the salivary glands as well as by the gastric, pulmonary and intestinal mucosa. Bile acids and colipase form micellar complexes with the lipids and bind lipase on the substrate water interface. Determination of Lipase is used for investigation of pancreatic disorders. In acute pancreatitis the lipase concentrations rise 2-50 fold the upper reference limit within 4-8 hours after beginning of abdominal pain peaking at 24 hours and decreasing within 8-14 days. Elevated Lipase values can also be observed in chronic pancreatitis and obstruction of the pancreatic duct.

Principle:

Pancreatic Lipase catalyses the hydrolysis of Triolein, in the presence of colipase, to form monoglycerides and fatty acids. The rate of decrease in turbidity measured at 340nm, is proportional to the lipase activity. The activities of other lipases in the serum are inhibited by the cholic acid salts in the reagent.

$$\text{Triolein} + 2H_2O \xrightarrow{\textit{Lipase}} \text{Monoglyceride} + 2\ \text{Oleic Acid}$$

Normal Reference Values:

Serum : up to 190U/L.

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	**10 ml**	**25 ml**	**75 ml**
L1 : Lipase Reagent	10 ml	25 ml	75 ml
C : Calibrator (for 1 ml)	1 No.	1 No.	1 No.

Storage/Stability

Contents are stable at 2-8^{0}C till the expiry mentioned on the labels. Do not freeze.

Reagent Preparation

The Lipase Reagent is ready to use.

Calibrator : Reconstitute the calibrator with 1 ml of D.W. Allow to stand for 10 mins with occasional mixing. The reconstituted control is stable for at least 7 days when stored at 2-8^{0}C and for at least 4 weeks when stored at -20^{0}C. Do not repeatedly thaw and refreeze.

Sample material

Serum. Free from Hemolysis.
Lipase is reported to be stable in serum for 5 days at 2-8^{0}C.

Procedure

Wavelength/Filter : 340 nm
Temperature : 37^{0}C
Light path : 1 cm

Pipette into clean dry test tubes labelled as Calibrator (C) and Test (T)

Addition Sequence	**C** (ml)	**T** (ml)
Lipase Reagent (L1)	1.0	1.0
Calibrator (C)	0.04	-
Sample	-	0.04

Mix well and incubate at 37^{0}C for 4 mins. and read the initial absorbance A_1 for the Calibrator (C) and Test (T). Read another absorbance A_2 of the Calibrator and Test after exactly 5 mins. Calculate the change in absorbance ΔA for both the Calibrator and Test.

For Calibrator $\Delta AC = A_2C - A_1C$
For Test $\Delta AT = A_2T - A_1T$

Calculations

$$\text{Lipase in U/L} = \frac{\Delta AT}{\Delta AC} \times \text{Conc. of Calibrator}$$

Linearity

The procedure is linear upto 700 U/L. If values exceed this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

Notes

Reagents such as Cholesterol / Triglycerides / HDL / LDL contain High Concentrations of detergents and hydrolysing enzymes, cross contamination from such reagents should be avoided.

All glassware / tips and cuvettes being used for the test should be throughly cleaned.

Samples having a very high activity show a very low initial absorbance as most of the substrate is consumed prior to the start of measurement. If this is suspected then dilute the sample and repeat the assay.

Do not use the Lipase reagent if the initial absorbance is below 1.000 at 340 nm against D.W.

In rare cases some sera may give an increase in absorbance, the Lipase activity of these samples usually falls within the normal range.

Components from human origin in the calibrator have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

References
Ziegenhorn J., et al. Clin.Chem.1979; **25**:1067.
Teitz NW et al. Clin. Chem.1993; **39**:746-756.
Lott,J. A., et al. Clin. Chem. 1986:**32**:1920
Tietz Textbook of Clinical Chemistry.3rd ed.: W.B Saunders Company; 1999.p.689-708.

System Parameters

Reaction	:	Fixed Time with Std.
Wavelength	:	340 nm
Zero Setting	:	Distilled Water
Incub. Temp.	:	37°C
Incub. Time	:	---
Delay Time	:	240 sec.
React. Time	:	300 sec.
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.04 ml
Reagent Vol.	:	1.00 ml
Standard	:	See Calibrator value
Factor	:	---
React. Slope	:	Decreasing
Linearity	:	700 U/L
Units	:	U/L

Coral Clinical Systems

Gitanjali, Dr. Antonio Do Rego Bagh,
Bambolim Complex P.O. GOA - 403 202, INDIA

LIP(UV).01(P)