LIPASE KIT

(Colorimetric Method)

(For veterinary invitro diagnostic use only)

INTENDED USE

QUADRAPED™ Lipase kit is used for the determination of Lipase Activity in serum / plasma.

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

In the presence of colipase and bile acids lipase splits the synthetic substrate (1,2-O-Dilauryl-rac-glycero-3-glutariacid (6-methyl-resorufin-ester) to glycerol and methylresorufin. The rate of methylresorufin formation, measured photometrically is propotional to the catalytic concentration of lipase present in the sample.

EXPECTED VALUES

Species	Lipase (U/L)
Dog	0-500
Cat	0-200
Cow	0-150
Horse	0-200
Pig	0-150
Sheep	0-150
Goat	0-150
Rabbit	0- 50
Buffalo	0-150

It is recommended that each laboratory establish its own range as reference ranges may vary between laboratories.

PRESENTATION

REF	1126150010
Pack Size	10 ml
L1 Buffer Reagent	8 ml
L2 Substrate Reagent	2 ml
C Calibrator (0.5 ml)	1 No.

COMPOSITION

Buffer Reagent : Goods Buffer (pH 8.0), Deoxycholate Taurodeoxycholate, Calcium Chloride, Colipase.

Substrate Reagent : Tartrate Buffer (pH 4.0), Taurodeoxycholate, Color substrate.

Lipase Calibrator : Lipase calibrator concentration is stated on the vial label.

STORAGE /STABILITY

The sealed reagents are stable up to the expiry date stated on the label, when stored at 2-8^{0}C, protected from light. Do not freeze.

REAGENT PREPARATION

The Lipase Reagents are ready to use.

Calibrator : Reconstitute the calibrator with 0.5 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 re-freeze.

SAMPLE MATERIAL

Serum or Plasma. Lipase is reported to be stable in serum, EDTA/ Heparinised plasma for 5 days at 2-8^{0}C.

SAMPLE WASTE AND DISPOSAL

Do not reuse the reagent containers, bottles, caps or plugs due to the risks of contamination and the potential to compromise reagent performance.
Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
Handle specimens, solid and liquid waste and test components in accordance with local regulations and NCCLS guidelines M29, or other published biohazard safety guidelines.

MATERIALS REQUIRED BUT NOT PROVIDED

Photometer analyzer with standard thermostatic cuvette holder, micropipette and appropriate laboratory equipment.

PROCEDURE

Wavelength / Filter : 580 nm
Temperature : 37^{0}C
Light path : 1 cm

Pipette into clean dry test tubes labelled as Blank(B), Calibrator (C), and Test (T).

Addition Sequence	B	S	T
Buffer Reagent	1000 µL	1000 µL	1000 µL
Calibrator	-	20 µL	-
Sample	-	-	20 µL
Distilled Water	20 µL	-	-
Mix carefully (do not vortex); incubate for 1-5 minutes at 37°C. Then add			
Substrate Reagent	250 µL	250 µL	250 µL

Mix well and incubate at 37^{0}C for 2 mins, and read the initial absorbance A_1 for the Blank, Calibrator and Test. Read another absorbance A_2 of the Blank, Calibrator and Test after exactly 2 mins. Calculate the change in absorbance ΔA for the Blank, Calibrator and the Test.

CALCULATIONS

$$\text{Lipase in U/L} = \frac{\Delta A \text{ Test} - \Delta A \text{ Blank}}{\Delta A \text{ Calibrator} - \Delta A \text{ Blank}} \times \text{Conc. of Calibrator}$$

QUALITY CONTROL

The following process is recommended for QC during the assay of Lipase. Define and establish acceptable range for your laboratory.

1. Two levels of control (Normal and Abnormal) are to be run on a daily basis.
2. If QC results fall outside acceptance criteria, re-calibration may be necessary.
3. Review QC results and run acceptance criteria following a change of reagent lot.

SPECIFIC PERFORMANCE CHARACTERISTICS

LOD: 2.5 U/L
LOQ: 5 U/L
Lower Limit: 2.5 U/L
Higher Limit: 300 U/L

If the value exceeds this limit, dilute the sample with normal saline (0.9% NACl) and repeat the assay. Calculate the value using proper dilution factor.

Interferences:
Sample when spiked with interferent such as upto 10 mg/dl Bilirubin, 200 mg/dl haemoglobin does not affect the ability of the kit to determine the Lipase concentration.

Precision:

Within run

Within run	n	Mean	SD	% CV
Sample 1	10	37.1	0.87	2.35
Sample 2	10	77.0	1.76	2.29
Sample 3	10	245.1	0.68	0.28

Between run

Between run	n	Mean	SD	% CV
Sample 1	10	37.2	0.83	2.24
Sample 2	10	76.3	1.71	2.25
Sample 3	10	245.3	0.75	0.31

Method comparison:
Comparative studies were done to compare our reagent with another commercial Lipase Assay. No significant differences were observed. Details of the comparative studies are available on request.

NOTE

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.
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. Do not use deteriorated or leaking reagents.

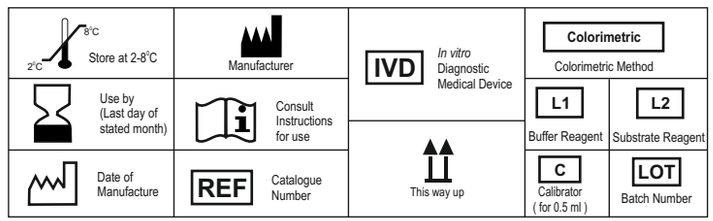
REFERENCES

1. Mc Neely, M. ; Lipase. Kaplan, A. *et al.;* Clin. Chem. The C.V. Mosby Co. St. Louis, Toronto. Princeton 1984, 1130-1135.
2. Burtis, A., et al. ; Tietz Textbook of Clinical chemistry, 3rd ed AACC.
3. Neumann, U., *et al*., Methods of Enzymatic Analysis, Vol 4,3rd ed.
4. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, Kenneth S. Latimer, ISBN Jane Wardrop , 6th Edition – 2010.
5. Clinical Biochemistry of Domestic Animals, Sixth Edition, 2008 by Kaneko J.J., Harvey J.W. & Bruss M.L.
6. Data on file : Coral Clinical Systems.

System Parameters

Reaction	: Fixed Time with Std.	**Interval**	: 120 Secs.
Wavelength	: 580 nm	**Sample Vol.**	: 20 µL
Zero Setting	: Reagent Blank	**Reagent 1 Vol.**	: 1000 µL
		Reagent 2 Vol.	: 250 µL
Incub. Temp.	: 37^0 C	**Standard**	: See Calibrator Value
Incub. Time	: ---	**Factor**	: ---
Delay Time	: 120 Sec.	**React. Slope**	: Increasing
Read Time	: 120 Sec.	**Linearity**	: 300 U/L
No. of read.	: —	**Units**	: U/L

SYMBOL KEYS



Manufactured by:

Coral Clinical Systems

A Division of Tulip Diagnostics (P) Ltd.

BUILDING E, PLOT NO. M-46/47, PHASE III B, VERNA INDUSTRIAL ESTATE, VERNA, GOA-403 722, INDIA.
REGD. OFFICE : GITANJALI, TULIP BLOCK, DR. ANTONIO DO REGO BAGH, ALTO SANTACRUZ, BAMBOLIM COMPLEX P.O., GOA-403 202, INDIA.

QLIP2/0625/VER-01