LIQUIZYME

LIPASE

(Methyl Resorufin Method)

BEACON

Code	Product Name	Pack Size
LS036A	Liquizyme Lipase	25 ml
LS036B	Liquizyme Lipase	50 ml

Intended Use
Diagnostic reagent for quantitative *in vitro* determination of Lipase in human serum and Plasma.

Clinical Significance
Lipases are enzymes which hydrolyze glycerol ester of long 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 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 to 2-50 fold to upper reference limit within 4-8 hours after begin of abdominal pain peaking at 24 hours and decreasing within 8 to 14 days. Elevated lipase values can also be observed in chronic pancreatitis and obstruction of the pancreatic duct.

Principle
Enzymatic color test.
The colorimetric substrate 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin)-ester is cleaved by pancreatic lipase and the resulting dicarboxilic acid ester is hydrolysed under the alkaline test condition to yield the chromophore methylresorufi. The kinetic of color formation at 580 nm is monitored and it is prooprtional to lipase activity in sample.

Reagent Composition
Reagent 1 : Lipase Reagent 1
Bicine Buffer : >40 mmol/l
Colipase : >0.98 mg/l
Na-Deoxycholate : >1 mmol/l
Calcium Chloride : >8 mmol/l

Reagent 2 : Lipase Reagent 2
Buffer : >8 mmol/l
Taurodeoxyl-Cholate : >8 mmol/l

Reagent 3 : Lipase Calibrator
Refer vial label for concentration

Reagent Preparation
Reagents are liquid, ready to use.

Stability And Storage
The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.
Reagent R2 is a microemulsion. Therefore, a slight apparent precipitation could occur, showing a light red deposit on the bottom of vial. It is a normal behavious and it is recommended to resuspend solution before analysis with a mild shaking.

Specimen Collection And Handling
Use serum, Plasma (hepairin, EDTA). It is recommended to follow NCCLS procedures (or similar standardized conditions).

Stability In Serum / Plasma :
7 days : at 4 – 8°C
1 year : at -20°C
Discard contaminated specimens.

Calibration
Calibration with the Lipase calibrator provided in the kit is recommended.

Quality Control
It's recommended to run normal and abnormal control sera to validate reagent performance.

Unit Conversion
U/l x 0.017 = µkat/l

Expected Values
Serum
at 37°C : Up to 60 U/L (=1.0 µkat/l)

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

Performance Data
Data contained within this section is representative of performance on Beacon system. Data obtained in your laboratory may differ from these values.

Limit of quantification : 3 U/L
Linearity : 300 U/L
Measuring range : 3 – 300 U/L

Precision

Intra-assay precision Within run (n=20)	Mean (U/L)	SD (U/L)	CV (%)
Sample 1	44	1.34	3.07
Sample 2	73	1.50	2.04

Inter-assay precision Run to run (n=20)	Mean (U/L)	SD (U/L)	CV (%)
Sample 1	38.73	1.11	2.87

Comparison
A comparison between Liquizyme Lipase (Methyl Resorufin Method) (y) and a commercially available test (x) using 20 samples gave following results:
y = 1.044x - 0.604 U/L
r = 0.995

Interferences
Following substances do not interfere:
Haemoglobin upto 4.5 g/l, bilirubin up to 40 mg/dl, triglycerides up to 1000 mg/dl.

Warning And Precautions

For *in vitro* diagnostic use. To be handled by entitled and professionally educated person.

Reagents 1 is not classified as dangerous. It contains less than 0.1% sodium azide, which is classified as very toxic and dangerous substance for environment.

Reagent 2 of the kit contains less than 5% propan-1-ol.

Waste Management

Please refer to local legal requirements.

Assay Procedure (Kinetic)

Wavelength : 580 nm

Cuvette : 1 cm

Addition Sequence	Calibrator	Sample
Reagent 1	1000 µl	1000 µl
Calibrator	20 µl	-
Sample	-	20 µl
Mix carefully (do not vortex) and incubate at 37°C for 1-5 min. then add		
Reagent 2	250 µl	250 µl

Mix immediately and read first absorbance of thest exactly at 120 sec. and second and third absorbance at an interval of 60 sec. At 580 nm. Determine the mean change in absorbance per minute (ΔA/min.)

Assay Procedure (Fixed Time)

Wavelength : 580 nm

Cuvette : 1 cm

Addition Sequence	Calibrator	Sample
Reagent 1	1000 µl	1000 µl
Calibrator	20 µl	-
Sample	-	20 µl
Mix carefully (do not vortex) and incubate at 37°C for 1-5 min. then add		
Reagent 2	250 µl	250 µl

Mix immediately and read first absorbance of thest exactly at 120 sec(A1). and second absorbance after exactly 120 sec(A2). Later Determine the mean change in absorbance as per ΔA = (A2-A1)

Calculation

For Kinetic:

$$\text{Lipase in U/L} = \Delta\text{ Abs./min} \times \text{Factor generated by using Calibrator}$$

For Fixed Time:

$$\text{Lipase in U/L} = \frac{\Delta\text{Abs.(A2-A1) of Sample}}{\Delta\text{Abs. (A2-A1)of Calibrator}} \times \text{Calibrator Concentration}$$

Applications for automatic analysers are available on request.

Assay Parameters For Photometers

Mode	Kinetic	Fixed Time
Wavelength 1 (nm)	580	580
Sample Volume (µl)	20	20
Reagent 1 Volume (µl)	1000	1000
Reagent 2 Volume (µl)	250	250
Lag time (sec.)	120	120
Kinetic Interval (sec.).	60	120
No. of Interval	2	-
Calibrator Value	See on Vial	See on Vial
Reaction temp. (°C)	37	37
Reaction Direction	Increasing	Increasing
Normal Low (U/L)	-	-
Normal High (U/L)	60	60
Linearity Low (U/L)	3	3
Linearity High (U/L)	300	300
Blank with	Reagent	Reagent
Unit	U/L	U/L

References

1. Lorentz K Lipase. In: Thomas L, editor, Clinical laboratory diagnostics. 1st ed. Frankfurt; TH-Books Verlagsgesell schaft; 1998.p.95-7.
2. Moss DW, Henderson AR. Digestive enzymes of pancreatic orgin. In: Burtis CA, Ashwood ER, editors, Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B.Saunders Company; 1999.p.689-708.
3. Tietz N, Shuey DF. Lipase in serum - the elusive enzyme; an overvies. Clin Chem 1993;39:746-56.

Symbols Used On Labels

REF	Catalogue Number		Manufacturer
	See Instruction for Use	LOT	Lot Number
CONT	Content		Storage Temperature
	Expiry Date	IVD	In Vitro Diagnostics

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