LIPASE
Multi-Purpose Liquid Reagent

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Cat. No.	Quantity	Reagent	Storage
GL537L	4 x 10 ml	LIPASE - R1	2-8°C
	1 x 8 ml	LIPASE - R2	

INTENDED USE:

In Vitro Diagnostic reagent pack for the quantitative determination of Lipase in serum and plasma.

SUMMARY AND EXPLANATION: [1-11]

Lipases are glycoproteins with a molecular weight of 47000 daltons. They are defined as Triglyceride hydrolases which catalyse the cleavage of Triglycerides to diglycerides with subsequent formation of monoglycerides and fatty acids. In addition to α-amylase, pancreatic lipases have for many years been undeniably the most important clinical chemistry parameters for the differential diagnosis of diseases of the pancreas. The lipase activity determination has gained increasing international recognition because of its high specificity and rapid response. After acute pancreatitis the lipase activity increases within 4-8 hours, reaches a peak after 24 hours and decreases after 8 to 14 days. However, there is no correlation between the lipase activity determined in serum and the extent of damage to the pancreas. Numerous methods have been described for the determination of lipase which determine the decrease in substrate turbidimetrically or nephelometrically, or determine degradation products. This method is based on the cleavage of a specific chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methyl-resorufin) ester emulsified with bile acids. The pancreatic enzyme activity is determined by the combination of bile acid and colipase used in this assay. Virtually no lipase activity is detected in the absence of colipase. Colipase only activates pancreatic lipase, but not other lipolytic enzymes found in serum. The high amounts of cholates ensure that the esterases present in the serum do not react with the chromogenic substrate due to the highly negative surface charge.

PRINCIPLE OF THE TEST: [8]

The measurement of serum Lipase is based on a kinetic colourimetric assay. The series of reactions involved in the enzymatic direct lipase determination is as follows:

1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methyl-resorufin) ester $\xrightarrow{\text{Lipase}}$ 1,2-O-dilauryl-rac-glycerol + glutaric acid-(6-methyl-resorufin) ester

glutaric acid-(6-methyl-resorufin) ester $\xrightarrow{\text{Spontaneous Decomposition}}$ glutaric acid + methylresorufin

The rate of methylresorufin formation, measured photometrically, is proportional to the catalytic concentration of lipase present in the sample.

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only - For Professional Use Only
Carefully read instructions for use. Deviations from this procedure may alter performance of the assay.

Components Colour and Appearance:
Reagent 1: Clear, colourless liquid.
Reagent 2: Turbid Orange Coloured Micro-emulsion.
Any significant changes could indicate that the assay might be compromised. Discard Reagent 2 if turning red. Refer to Laboratory's QC program for actions to be taken. In case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety precautions:
This product is not hazardous under EU specifications. Material Safety Data Sheet is available upon request.

Handling precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not Freeze Reagents.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results.
- Refer to local legal requirements for safe waste disposal.

INSTRUMENTS:
Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	Ingredients	Concentration in Tests
Reagent 1	TRIS pH 8.3	40 mmol/L
	Colipase	≥ 1 mg/L
	Desoxycholate	1.8 mmol/L
	Taurodesoxycholate	7.2 mmol/L
Reagent 2	Tartrate pH 4.0	15 mmol/L
	Lipase Substrate	≥ 0.7 mmol/L
	Calcium Chloride ($CaCl_2$)	0.1 mmol/L

REAGENT PREPARATION AND STABILITY:

Reagent 1 and 2 are ready for use. Stability, after opening, is 90 days at 2-8°C if stored tightly capped.
Before use, mix reagent by gently inverting each bottle.
If stored and handled properly, unopened component is stable until expiry date stated on the label.

TYPE OF SPECIMEN:

Use serum *or* plasma with sodium citrate, EDTA or heparin as specimen[14].

Do not use EDTA, oxalate, fluoride or citrate as this can lead to decreased results (inhibition of lipase activity).
It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. Serum/Plasma should be separated from cells within 2 hours after collection.
Stability: up to 2 days at 2-8°C.

TEST PROCEDURE:
Materials required but not supplied:

Description	Catalog. No.	Description	Catalog. No.
General Chemistry Calibrator	GL983		
General Chemistry Control Level 1	GL922		
General Chemistry Control Level 2	GL932	Spectrophotometer or colorimeter at 580nm	N/A
General Laboratory Equipment	N/A		

Assay procedure:
Wavelength: λ: 578 nm
Temperature: 37°C
Optical path: 1 cm light path.

	Blank	Calibrator	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	----	----	10 µl
Calibrator	----	10 µl	----
Gently mix and Incubate at 37°C for 3 minutes.			
Reagent 2	200 µl	200 µl	200 µl
Gently mix and Incubate at 37°C - Measure the Optical Density after 2 minutes. Read the Δ absorbance per minute of calibration blank and sample.			

Calibration:
Using recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for Q.C. should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's Q.C. Programme.
Controls should be assayed:
- Prior to reporting patient results.
- Following any maintenance procedure.
- At intervals established by the Laboratory Q.C. Programme.

CALCULATION:

$$\text{Concentration of Lipase} = \frac{\Delta\text{Absorbance Test}}{\Delta\text{Absorbance Calibrator}} \times \text{Concentration of Calibrator}$$

(Conversion Factor: Qty in µkat/l = U/l x 0.017).

EXPECTED VALUES[13]:

	U/l	µkat/l
Adults	5.6-51.3	0.0952-0.8721

These values should be used as a guide only. Each laboratory should establish its own reference range. Lipase results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:
Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity: This assay is linear up to 250 U/l.
For samples with a higher activity, dilute the sample 1/10 with 0.9% NaCl (9 g/L) and multiply the result by 10.

Interfering substances:
Bilirubin (mixed isomers): No interference up to 20 mg/dl Bilirubin.
Haemolysis: No interference up to 150 mg/dl Haemoglobin.
Triglycerides: Less than 10% interference up to 300 mg/dl Triglycerides

Sensitivity:
The Lowest Detectable Level was estimated at 5 U/l.

Precision:

Within Run N = 21	Mean (U/l)	SD	% CV	Between Run N = 11	Mean (U/l)	SD	% CV
Level 1	119	4.13	3.34	Level 1	119	5.43	4.54
Level 2	215	5.97	2.78	Level 2	215	10.7	5.02

Method Comparison:
Using 100 samples, a comparison, between this Lipase test (y) and another commercially available test (x), gave the following results:

y = 0.50054x + 3.9443	r = 0.997

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SYMBOLS:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	CE	CE Mark - Device comply with the Directives 98/79/EC
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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