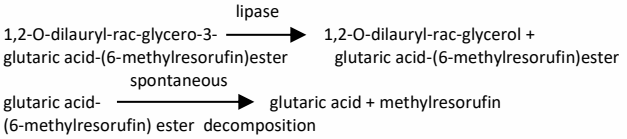
LIPASE

ENZYMATIC

Intended use:
Enzymatic assay for the in vitro quantitative determination of Lipase in human serum and plasma.

Summary:
Lipases are glycoproteins with a molecular weight of 47000 Daltons. They are defined as triglyceride hydrolases which catalyze the cleavage of triglycerides to diglycerides with subsequent formation of monoglycerides and fatty acids. In addition to a-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, researches 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-methylresorufin) ester emulsified with bile acids. The pancreatic enzyme activity is determined specifically by the combination of bile acid and colipase used in this assay. Virtually no lipase activity is detected in the absence of co-lipase. Colipase only activates pancreatic lipase, but not other lipolytic enzymes found in serum. The high amount of cholates ensure that the esterases present in the serum do not react with the chromogenic substrate due to the highly negative surface charge.

Test principle:
Enzymatic colorimetric assay;
• Sample and addition of R1 (buffer/colipase/cholate)
• Addition of R2 (emulsion/chromogenic substrate/cholate) and start of reaction:



The chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester is cleaved by the catalytic action of alkaline lipase solution to form 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6-methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. The colour intensity of the red dye formed is directly proportional to the lipase activity and can be determined photometrically.

Reagent concentration:

R1:

Buffer/colipase/cholate BICIN buffer*: pH 8.0	50 mmol/l
colipase (porcine pancreas)	1mg/l
Na-deoxycholate	1.6 mmol/l
calcium chloride	10 mmol/l
detergent	
preservative	

R2:

Emulsion/chromogenic substrate/cholate	
Tartrate buffer pH 4.0	10 mmol/l
1,2-O-dilauryl-rac-glycero-3 glutaric acid-(6-methylresorufin) ester	0.27 mmol/l
taurodeoxycholate	8.8 mmol/l
detergent preservative	

Preparation and Stability:
R1: Ready for use.
R2: Ready for use.
Unopened kit components: Up to the expiration date at +2°C to +8°C
Onboard stability:

R1: 28 days opened and refrigerated on the analyser
R2: 28 days opened and refrigerated on the analyser
Store protected from light after opening.

Specimen:
Collect serum using standard sampling tubes
Li-, Na- or NH4- heparin plasma.
Stability: 7 days at +20°C to +25°C
7 days at +4°C to +8°C
1 year at –20°C

EDTA-, oxalate-, fluoride- or citrated plasma lead to decreased results (inhibition of lipase activity).
Centrifuge samples containing precipitate before performing the assay.

Notes:
For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.

Limitations - interference:
Criterion: Recovery within ±10% of initial values.
(Only Hitachi): Use the evasion software with SMS for the individual assays in order to avoid cuvette carry-over of triglycerides, cholesterol, HDL cholesterol plus and LDL-cholesterol plus to lipase and probe carry-over of triglycerides and cholesterol to lipase as well as from lipase to calcium.
Icterus: No significant interference up to an index I of 60 (approximate bilirubin concentration: 60 mg/dl). Hemolysis: No significant interference up to an index H of 500 (approximate haemoglobin concentration: 500 mg/dl).
Lipemia (Intralipid): No significant interference up to an index L of 1000 (approximate triglycerides concentration: 2000 mg/dl).
There is poor correlation between turbidity and triglycerides concentration.

Testing procedure:
Applications for automated systems are available on request.
Materials provided
• Working solutions as described above
Additional materials required
• Calibrators and controls as indicated below
• 0.9% NaCl

Manual procedure (reagent start)	
Wavelength:	580nm (560nm-580nm)
Temperature:	+37°C
Cuvette:	1 cm light path
Zero adjustment:	Air or water.
	Serum /Plasma
R1	800 µl
Sample	16 µl
Mix and incubate for 5 minutes.	
R2	200 µl
Mix, read initial absorbance after 2 minute. Read absorbance again after 3, 4 and 5 min. Calculate A/min.	
Calculation: A/min Sample A/min Calibrator X Calibrator conc. = Lipase activity (U/l)	

Measuring /reportable range:
Measuring range: 3 - 300 U/l (0.05-5.00 µkat/l)
Determine samples with lipase activity > 300 U/l via the rerun function. Manually dilute the original samples having activities > 3300 U/l (55.00 ukat/l) with 0.9% NaCl or distilled/deionized water (e.g. 1 + 50). Multiply the result by the appropriate dilution factor (e.g. 51).
Manually dilute samples with higher activities with 0.9% NaCl or distilled/deionized water (e.g. 1 + 10). Multiply the result by the appropriate dilution-factor (e.g. 11).
Provisional expected values:
Adults: < 60 U/l (<1.00µkat/l)
In order to relate findings to the lipase reference range obtained using the turbidimetric method, multiply the results (U/l or µkat/l) by the factor 3.2 to obtain an approximate comparison.
Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

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Analytical sensitivity (lower detection limit):
Detection limit: 3 U/l (0.05 µkat/l)
The lower detection limit represents the lowest measurable lipase activity that can be distinguished from zero.

Imprecision:
Reproducibility was determined using controls in an internal protocol. The following results were obtained:

Within run			
Sample	Mean (U/l)	SD (U/l)	CV (%)
Control serum 1	34	0.39	1.16
Control serum 2	71	0.51	0.72
Control serum 3	58	0.39	0.67
Day/day			
Sample	Mean (U/l)	SD (U/l)	CV (%)
Control serum 1	35	0.23	0.65
Control serum 2	63	0.55	0.87
Control serum 3	72	0.90	1.26

Method comparison:
A comparison of the BIOANALYTIC LIPASE Conc. (y) with a turbidimetric assay gives the following result:
y =0.304 x + 6.563; r = 0.986.

Quality Control:
Control Serum:
BIOCON N 5 x 5 ml # B10814
BIOCON P 5 x 5 ml # B10817
The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:
S1: 0.9% NaCl
S2: BIOCAL H 5 x 3 ml #B11895

Calibration frequency:

It is suggested to use Calibrator products produced by Bioanaliytic. It is
suggested to use supplementary calibrator (pure water or 0.9% NaCl) to
conduct 2-point calibration. The calibration curve is formed automatically.
When lot number is changed or QC is invalid, calibration shall be conducted
again. Recalibrate the assay every 30 days under ideal conditions, or when the following occur:
Change in reagent lot or significant shift in control values;
Major preventative maintenance was performed on the analyser or a critical
part was replaced(Halogen Lamp)

Order information (Cat No.) :

CC500	B21225	B25226	B30226	B33226	B37225
OL500	B21226	B27225	B31225	B34225	B37226
KL500	B22225	B27226	B31226	B35225	B80225
SH500	B24225	B28225	B32225	B35226	B80226
AB500	B24226	B28226	B32226	B36225	B80227
CR500	B25225	B30225	B33225	B36226	B80228

Literature:
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fluid. DG Klinische Chemische Mitteilungen 1995;26:207-224.
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Manufacturer
Diaclinica Diagnostik Kimya.San.Tic.Ltd.Şti
Adress : İkitelli O.S.B Mutsan San.Sit. M4 Blok No:17-19 Başakşehir/İSTANBUL
Tel:+90(212) 549 33 88- Fax:+90 (212) 549 55 50
Web :www.diaclinica.com

SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

ISO 9001:2015
ISO 13485:2016