LIPASE

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REF No: 06R55-22 2856 Tests
REF No: 06R55-23 1540 Tests

FOR USE WITH
ARCHITECT

Diagnostic reagent for determination of Lipase concentration.
Liquid. Dual reagent. Store at +2/+8°C. For in Vitro Diagnostic use (IVD). **Do not freeze.**
Products with 06R55-22/06R55-23 Ref Number are produced for Abbott Architect Biochemistry Autoanalyzer Series

Changes made in the instructions for use are marked as grey.

INTENDED USE

Archem lipase assay is used for the quantitative in vitro determination of lipase activity in human serum or plasma by autoanalyzers in a clinical laboratory setting.

GENERAL INFORMATION

Human pancreatic lipase (EC 3.1.1.3; triacylglycerol acylhydrolase) is a single-chain form having 48 kDa molecular weight and approximately 5.8 isoelectric point. The lipase gene is located on chromosome 10. To show full catalytic activity and highest specificity, it requires bile salts and the presence of a cofactor called colipase, a small molecular weight protein of 10 kDa secreted by pancreatic acinar cells.[1] Human lipase can be fully activated by colipases from other species (e.g. porcine colipase) in vitro; this property is used in analytical formulations of the lipase assay.[2]

Lipases (including lipoprotein lipase) are defined as enzymes that hydrolyze glycerol esters of long-chain fatty acids. Only ester bonds at carbons 1 and 3 (α-positions) are attacked and the reaction products contain 2 mol of fatty acids and 1 mol of 2-acylglycerol (β-monoglyceride) per mole of substrate. β-monoglyceride is resistant to hydrolysis, probably due to steric hindrance, but can spontaneously isomerize to the α-form (3-acylglycerol). This isomerization allows the third fatty acid to be cleaved at a much slower rate.[1] The control of lipase secretion and associated factors appears to be driven by the contents of the gastrointestinal lumen, particularly by the presence of acid or digested proteins and fats in the duodenal lumen. Secretion of cholipase, bile acids and lipase is driven by the release of cholecystokinin and secretin.[3] Most of the lipase activity found in serum originates from pancreatic acinar cells, but some is secreted by the gastric and intestinal mucosa.

The concentration of lipase in the pancreas is about 5000 times higher than in other tissues, and the concentration difference between pancreas and serum is about 20,000 times.[1,4]

Lipase is a small enough molecule to be filtered through the glomeruli, but is completely reabsorbed by the renal tubules and is not physiologically detectable in urine. Evidence suggests that pancreatic lipase may exist in at least two isoforms, but the exact nature of these forms is unknown. Complete absence of lipase has been reported in the literature. This congenital absence results in fat malabsorption and severe steatorrhea. Lipase has much less tissue distribution than P-type amylase and therefore its elevation in serum is less associated with non-pancreatic disease states.[1]

Serum lipase measurement is the recommended laboratory test for the diagnosis of acute pancreatitis. Clinical sensitivity ranges from 80 to 100% depending on the diagnostic predictive value chosen, while clinical specificity ranges from 85 to 100% depending on the patient population studied. After an episode of acute pancreatitis, serum lipase activity increases within 4-8 hours, peaks at approximately 24 hours and decreases within 7-14 days. In this case, increases between 2 and 50 times the upper reference limit (URL) have been reported. However, the increase in serum lipase activity is not always proportional to the severity of the attack. In addition, in pediatric acute pancreatitis, a serum lipase activity greater than seven times the URL within 24 hours of the attack was associated with a 7.1-fold risk ratio (95% confidence interval, 2.5 to 20.5) for developing severe pancreatitis.[1,5]

The diagnosis of acute pancreatitis is sometimes difficult because it must be differentiated from other acute intra-abdominal diseases with similar clinical manifestations, such as acute cholecystitis, perforated gastric or duodenal ulcer, intestinal obstruction or ruptured abdominal aortic aneurysm. Because treatment of other conditions mimicking pancreatitis typically involves surgery and surgical intervention is generally contraindicated in pancreatitis, accurate diagnosis of pancreatitis is vital.[1] Also for the diagnosis, in the absence of renal failure, an increase in serum lipase activity more than three times the URL is a more specific diagnostic finding than an increase in serum α-amylase activity.[6] In fact, the mean peak increase in lipase activity after acute pancreatitis is about

four times greater than that of amylase. Finally, lipase concentrations remain elevated longer than those of α-amylase, which is another advantage over α-amylase measurement in patients with delayed diagnosis.[1] Therefore, it is recommended that lipase replace α-amylase as the first diagnostic test for acute pancreatitis in the emergency department.[7] However, international practice differs in this respect.[8]

Serum lipase activity may be increased in patients with significantly reduced glomerular filtration rate (GFR). Thus, great caution is required in the interpretation of increased serum lipase values in the presence of chronic kidney disease. In a rare case, inaccuracies in serum lipase estimation have also been shown to be due to the presence of lipase macroforms consisting of IgG-bound enzyme, poorly filtered and excreted by the kidneys due to its large size, which would lead to elevated values if enzyme activity in the blood were measured. In addition, examination of the biliary tract by endoscopic retrograde pancreatography or opiate use (as it causes contraction of the sphincter of Oddi) may increase serum lipase activity.[1]

TEST PRINCIPLE

Enzymatic colorimetric measurement

Cleavage of the synthetic substrate 1,2-O-dilauryl-rac-glycerol-3-glutaric acid-(6'methylresorufin) ester in alkaline solution by the catalytic action of pancreatic lipase yields 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6'methylresorufin) ester. Decomposition of this intermediate product by self- hydrolyzing in alkaline medium leads to the formation of glutaric acid and methylresorufin with chromogenic properties.The absorbance value of this red colored product at 580 nm wavelength is directly proportional to the lipase activity in the sample.

1,2 – O – dilauryl – rak – glycero – 3 - glutaric acid – (6 – methylresorufin) esther $\xrightarrow{Lipase}$ 1,2 – O – dilauryl – rak – glycerol + glutaric acid – (6 – methylresorufin) esther

Glutaric acid – (6 – methylresorufin) esther $\xrightarrow{spontaneous\ decay}$ glutaric acid + methylresorufin

REAGENTS COMPONENTS

Reagent 1:

Tris buffer	: 40 mmol/L
Colipase	: ≥ 1 mg/L
Desoxycholate	: ≥ 1.8 mmol/L
Taurodesoxycholate	: ≥ 7.0 mmol/L

Reagent 2:

Tartrate buffer	: 15 mmol/L
Lipase substrate	: ≥ 0.70 mmol/L
Calcium ions	: ≥ 1 mmol/L

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[9]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. Multiple samples freezing and thawing should be avoided. The sample should be homogenized before testing.

Stability of the serum and plasma samples[21]:

7 days at +20/+25ºC
3 weeks at +2+8ºC
1 year at -20º

Unit Conversion:

U/L x 0.0167 = µkat/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Lipase Calibrator Set.

Lipase Calibrator Set (Lyophilized)
Ref.No: 06R56-01

Calibration stability is 15 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVAL / MEDICAL DECISION LEVELS

Adults[22] : ≤ 60 U/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[10,11]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[12]

The determined analytic measuring interval for Lipase is 2-400 U/L.

Detection Capability

Limit of Detection (LoD): 2 U/L

Limit of Quantitation (LoQ): 5 U/L

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[13]

Linearity

This method shows measurement linearity in the activities up to 400 U/L. Autoanalyzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.
For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.
Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[14]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[15]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Lipase have been given in the table 1 and 2 respectively.

Table 1. Lipase Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
31 U/L	0.48	1.56	80
224 U/L	4.63	2.06	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[16]

Table 2. Lipase Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
31 U/L	1.15	3.71	80
224 U/L	11.5	5.15	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[16]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablok equation:[17]
y= 1.096x – 4.45 IU/mL
r=0.989

Interference

Endogenous interferant and analyte concentrations that have been used in the Lipase scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[18,19]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Lipase interference scanning test is appropriate, is determined as ±10%.[20]

In Lipase test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interfering Substance and Concentration	Lipase Target (U/L)	N	Observed Recovery %
Hemoglobin 1260 mg/dL	31	3	97
Bilirubin 9.47 mg/dL	33	3	94
Lipemia 570 mg/dL	26	3	100

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[19]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium florid, iodoacetate, hydrochloride acid) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[19]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practice
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests