LIPC

cobas®

Lipase colorimetric

Enzymes

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03029590 322	Lipase colorimetric (200 tests)	System-ID 07 5900 7	COBAS INTEGRA 400 plus
10759350 190	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6	
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	System-ID 07 3718 6	
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7	
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	System-ID 07 7999 7	
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6	
12149443 160	Precipath U plus (10 × 3 mL, for USA)	System-ID 07 8000 6	
10171743 122	Precinorm U (20 × 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 × 5 mL)	System-ID 07 7997 0	
10171778 122	Precipath U (20 × 5 mL)	System-ID 07 7998 9	
10171760 122	Precipath U (4 × 5 mL)	System-ID 07 7998 9	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7	

English

New application

System information

Test LIP, test ID 0-052

Intended use

In vitro test for the quantitative determination of the catalytic activity of lipase (EC 3.1.1.3; triacylglycerol acyl-hydrolase) in human serum and plasma on COBAS INTEGRA systems.

Summary[1,2,3,4,5,6,7]

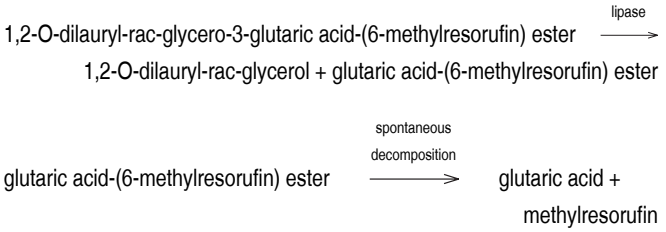
Lipases are glycoproteins with a molecular weight of 47000 daltons. They are defined as triglyceride hydrolases which catalyze the cleavage of triglycerides to diglycerides with subsequent formation of monoglycerides and fatty acids. In addition to α-amylase, pancreatic lipases have for many years been 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-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 colipase. Colipase only activates pancreatic lipase, but not other lipolytic enzymes found in serum. The high amount of cholates ensures that the esterases present in the serum do not react with the chromogenic substrate due to the highly negative surface charge.

Test principle[8,9,10,11]

Enzymatic colorimetric assay with 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester as substrate.

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. Addition of detergent and colipase increases the specificity of the assay for pancreatic lipase.



The color intensity of the red dye formed is directly proportional to the lipase activity and can be determined photometrically.

Reagents - working solutions

R1 BICIN[a)] buffer: 50 mmol/L, pH 8.0; colipase (porcine pancreas): ≥ 0.9 mg/L; Na-deoxycholate: 1.6 mmol/L; calcium chloride: 10 mmol/L; detergent; preservative

SR Tartrate buffer: 10 mmol/L, pH 4.16; 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester: 0.27 mmol/L; taurodeoxycholate: 8.8 mmol/L; detergent; preservative

a) BICIN = N,N-bis(2-hydroxyethyl)glycine

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	4 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable: Serum: collect serum using standard sampling tubes. Fresh serum is the

specimen of choice.
Plasma: Li-heparin plasma

Do not use calcium complexing anticoagulants such as EDTA, citrate, and fluoride.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability in serum:[12]	1 week at 15-25 °C
	1 week at 2-8 °C
	1 year at (-15)-(-25) °C
Stability in plasma:[13]	1 week at 15-25 °C
	1 week at 2-8 °C
	2 months at (-15)-(-25) °C

Materials provided

See "Reagents – working solutions" section for reagents.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	583/659 nm
Calc. first/last	40-46
Unit	U/L

Pipetting parameters

		Diluent (H_2O)
R1	80 µL	
Sample	2 µL	20 µL
SR	48 µL	
Total volume	150 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized manually against Roche reagent using the substrate-specific absorptivity, ε.

Quality control

Reference range	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U, Precipath U plus or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus analyzers).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 % of an initial value at a lipase activity of 60 U/L (1.00 µkat/L).

Icterus:[14] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[14] No significant interference up to an H index of 300 (approximate hemoglobin concentration: 186 µmol/L or 300 mg/dL).

Lipemia (Intralipid):[14] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[15,16]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[17]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

3-300 U/L (0.05-5.01 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 U/L (0.05 µkat/L)
Limit of Detection	= 3 U/L (0.05 µkat/L)
Limit of Quantitation	= 5 U/L (0.08 µkat/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a precision coefficient of variation of 20 %. It has been determined using low concentration of lipase samples.

Expected values[18]

Adults	13-60 U/L	(0.22-1.00 µkat/L)

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

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
PCCC Multi 1	46.4 (0.78)	0.64 (0.012)	1.4
PCCC Multi 2	97.8 (1.63)	1.46 (0.024)	1.5
Human serum 1	12.6 (0.21)	0.33 (0.006)	2.6
Human serum 2	47.4 (0.79)	0.63 (0.011)	1.3
Human serum 3	75.1 (1.25)	0.90 (0.015)	1.2
Human serum 4	139 (2.32)	1.59 (0.027)	1.1
Human serum 5	264 (4.41)	3.24 (0.054)	1.2

Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
PCCC Multi 1	46.4 (0.78)	0.86 (0.014)	1.8
PCCC Multi 2	99.5 (1.66)	1.98 (0.033)	2.0
Human serum 1	12.6 (0.21)	0.35 (0.006)	2.7
Human serum 2	47.4 (0.79)	0.95 (0.016)	2.0
Human serum 3	74.5 (1.24)	1.43 (0.024)	1.9
Human serum 4	138 (2.31)	2.74 (0.046)	2.0
Human serum 5	269 (4.49)	4.95 (0.083)	1.8

PCCC = PreciControl ClinChem

Method comparison

Lipase values for human serum and plasma samples obtained on a COBAS INTEGRA 400 plus analyzer with the COBAS INTEGRA Lipase colorimetric reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi MODULAR P analyzer (x).

Sample size (n) = 108

Passing/Bablok[19]	Linear regression
y = 1.01x - 0.643 U/L	y = 1.01x - 0.269 U/L
τ = 0.982	r = 0.999

The sample activities were between 4.93 and 293 U/L (0.08 and 4.89 µkat/L).

References

1 Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.

2 Keller H, ed. Klinisch-chemische Labordiagnostik für die Praxis, 2nd ed. Stuttgart/New York: Georg Thieme Verlag 1991:354-361.

3 Kazmierczak S, Catrou P, Van Lente F. Diagnostic accuracy of pancreatic enzymes evaluated by use of multivariate data analysis. Clin Chem 1993;39:1960-1965.

4 Steinberg WM, Goldstein SS, Davies ND, et al. Diagnostic assays in acute pancreatitis [Review]. Ann Intern Med 1985;102:576-580.

5 Panteghini M, Pagani F, Bonora R, et al. Diagnostic value of four assays for lipase determination in serum: A comparative reevalution. Clin Biochem 1991;24:497-503.

6 Tietz NW, Shuey DF. Lipase in serum - the elusive enzyme: An overview. Clin Chem 1993;39(5):746-756

7 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia PA: WB Saunders Company 1995;865.

8 Neumann U, Junius M, Batz HG, et al. New substrates for the optical determination of lipase. EP 207252 1987.

9 Borgström B. The action of bile salts and other detergents on pancreatic lipase and the interaction with colipase. Biochimica et Biophysica Acta 1977;488:381-391.

10 Gargouri Y, Julien R, Bois A, et al. Studies on the detergent inhibition of pancreatic lipase activity. J of Lipid Research 1983;24:1336-1342.

11 Leybold A, Junge W. Importance of colipase for the measurement of serum lipase activity. Adv Clin Enzymol 1986;4:60-67.

12 Guder W, Fonseca-Wollheim W, Heil O, et al. Maximum permissible transport and storage times for analysis of blood (serum, plasma), urine and cerebrospinal fluid. DG Klinische Chemische Mitteilungen 1995;26:207-224.

13 Data on file at Roche Diagnostics.

14 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

15 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

16 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

17 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

18 Junge W, Abicht K, Goldmann J, et al. Evaluation of the Colorimetric Liquid Assay for Pancreatic Lipase on Hitachi Analyzers in 7 Clinical Centers in Europe, Japan and USA. Clin Chem Lab Med 1999;37(Special Suppl):469.

19 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

LIPC

Lipase colorimetric

cobas®

Enzymes

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