LIPC

cobas®

Lipase colorimetric assay

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057982190	08057982500	Lipase colorimetric assay (200 tests)	System-ID 2085 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

LIP: ACN 20850

Intended use

Enzymatic in vitro test for the quantitative determination of lipase in human serum and plasma on **cobas c** systems.

Summary

Lipase measurements, performed with this assay in human serum and plasma, are used as an aid in the diagnosis and monitoring of various pancreatic conditions, particularly acute pancreatitis.

Lipases are triglyceride hydrolases which catalyze the cleavage of triglycerides into fatty acids and glycerol.[1,2] Most of the lipase activity found in serum derives from pancreatic acinar cells, but some is secreted by gastric and intestinal mucosa.[1,2] Human pancreatic lipase is a glycoprotein with a molecular weight of 45-48 kDa.[1,2,3] It is secreted into the duodenum through the duct system of the pancreas, and the concentration in blood is normally very low: the concentration gradient between pancreatic tissue and serum lipase is approximately 20,000-fold. Upon pancreatic injury, the pancreas starts to release the lipase into blood at higher amounts. This can occur in conditions such as acute pancreatitis, chronic pancreatitis, pancreatic cancer, or pancreatic duct obstruction. Therefore, the measurement of pancreatic lipase in blood can be used as an aid to diagnose acute pancreatitis and other pancreatic diseases.[2,3]

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.[4,5,6,7] 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.[2,4,5,6]

Lipase activity in serum can also be influenced by factors other than pancreatic disorders, such as kidney disease, intestinal ischemia, or certain medications.[1,2] Therefore, clinical interpretation of lipase levels should be done in conjunction with a comprehensive assessment of the patient's medical history, symptoms, and other diagnostic tests.

The hydrolyzation action of lipase can only take place when the substrate is present in an emulsified form and the rate of action depends on the free surface area of the substrate. Bile and co-lipase are thus essential for the activity of pancreatic lipase as bile helps emulsify fats, increasing their surface area for lipase action, and co-lipase enhances the binding and activity of lipase at the lipid-water interface.[1]

Numerous methods have been described for the determination of lipase which determine the decrease in substrate turbidimetrically or nephelometrically or determine degradation products.[1,3,8,9] The method of this assay 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[10,11,12,13]

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.

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

glutaric acid-(6-methylresorufin) ester $\xrightarrow{\text{spontaneous decomposition}}$ glutaric acid + methylresorufin

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

R3 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 R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

LIPC

Lipase colorimetric assay

H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.

Prevention:

P261	Avoid breathing mist or vapours.
P280	Wear protective gloves/ eye protection/ face protection.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	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
Plasma: Li-heparin plasma

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.

See the limitations and interferences section for details about possible sample interferences.

Stability in serum:[14]	7 days at 20-25 °C
	7 days at 4-8 °C
	1 year at -20 °C (±5 °C)

Freeze only once.

Stability in plasma:	1 week at 15-25 °C
	1 week at 2-8 °C
	2 months at -20 °C (±5 °C)

Freeze only once.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/570 nm		
Reagent pipetting		Diluent (H_2O)	
R1	60 µL	15 µL	
R3	36 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	1.5 µL	10	90
Increased	1.5 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - 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

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 4 weeks. 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 c systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

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

Limitations - interference

Criterion: Recovery within ± 6 U/L of initial values of samples ≤ 60 U/L and within ± 10 % for samples > 60 U/L.

Icterus:[15] 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:[15] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L or 100 mg/dL).

Lipemia (Intralipid):[15] 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.[16,17]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[18]

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 c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

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 activity 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 activity samples.

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

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity lipase samples.

Expected values[19]

Adults: 13-60 U/L (0.22-1.00 µkat/L*)

*calculated by unit conversion factor

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 analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[b)]	45.5	0.295	0.6
PCCC2[c)]	102	0.425	0.4
Human serum 1	6.59	0.230	3.5
Human serum 2	40.2	0.245	0.6
Human serum 3	94.4	0.445	0.5
Human serum 4	152	0.617	0.4
Human serum 5	250	0.866	0.3

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[b)]	45.5	0.498	1.1
PCCC2[c)]	99.3	1.08	1.1
Human serum 1	6.59	0.267	4.1
Human serum 2	40.2	0.368	0.9
Human serum 3	94.4	1.01	1.1
Human serum 4	142	1.57	1.1
Human serum 5	250	2.79	1.1

b) PreciControl ClinChem Multi 1

c) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Lipase values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 72

Passing/Bablok[20]	Linear regression
y = 1.013x + 0.718 U/L	y = 1.033x + 0.415 U/L
τ = 0.962	r = 0.998

The sample activities were between 3.29 and 261 U/L.

Lipase values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 71

Passing/Bablok[20]	Linear regression
y = 1.039x + 0.475 U/L	y = 1.027x + 0.689 U/L
τ = 0.975	r = 0.999

The sample activities were between 4.30 and 282 U/L.

Lipase values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 75

Passing/Bablok[20]	Linear regression
y = 0.980x + 0.265 U/L	y = 0.976x + 0.625 U/L
τ = 0.955	r = 1.000

The sample concentrations were between 5.06 and 288 U/L.

References

1 Pincus MR, McPherson RA, Bock J. Chemical Basis for Analyte Assays and Common Interferences. In: McPherson RA, Pincus MR, editors. Henry's Clinical Diagnosis and Management by Laboratory Methods, Elsevier, 24th edition, 2022, chapter 28, p. 453-466.e1.

2 Panteghini M. Serum Enzymes. In: Rifai N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 32, p. 350-350.e36.

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

4 Agrawal A, Parikh M, Thella K, et al. Acute pancreatitis with normal lipase and amylase: an ED dilemma. Am J Emerg Med 2016 Nov;34(11):2254.e3-2254.e6.

5 Ismail OZ, Bhayana V. Lipase or amylase for the diagnosis of acute pancreatitis? Clin Biochem 2017 Dec;50(18):1275-1280.

6 Rompianesi G, Hann A, Komolafe O, et al. Serum amylase and lipase and urinary trypsinogen and amylase for diagnosis of acute pancreatitis. Cochrane Database Syst Rev 2017 Apr 21;4(4):CD012010.

7 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.

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

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

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

11 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.

12 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.

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

14 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.

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

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

17 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.

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

19 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.

20 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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606