

Order information

REF	CONTENT	System-ID	Analyzers on which cobas c pack(s) can be used
05852625 190	Tina-quant Lipoprotein (a) Gen.2 (150 tests)	System-ID 07 7504 5	COBAS INTEGRA 400 plus COBAS INTEGRA 800
05852641 190	Preciset Lp(a) Gen.2 (5 × 1 mL)	System-ID 07 7546 0	
05852650 190	PreciControl Lp(a) Gen.2 Level Low (2 × 1 mL) Level High (2 × 1 mL)	System-ID 07 7544 4 System-ID 07 7545 2	
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0	

English

System information

Test LPA2, test ID 0-039

Intended use

In vitro test for the quantitative determination of lipoprotein (a) in human serum and plasma on COBAS INTEGRA systems.

Summary

Lipoprotein (a) (Lp(a)) is composed of an LDL-like particle to which the lipoprotein (a)-specific apolipoprotein (a) is bonded by a disulfide bridge. Apolipoprotein (a) is highly homologous to plasminogen. Lipoprotein (a) is a cholesterol-rich lipoprotein which is synthesized in the liver independently of triglycerides and is not subject to the influence of age or diet.¹

Several unrelated studies showed that Lp(a) is an independent prospective risk factor for coronary heart disease. However acceptance is limited due to the fact that it is difficult to compare Lp(a) results between different clinical studies and the assays used showed strong variations and miscellaneous standardization levels.^{2,3} Main problem for accurate detection of Lp(a) is the size polymorphism of apolipoprotein a (apo (a)). Levels of Lp(a) vary drastically among individuals and ethnic groups as the level is predominantly determined by the apo (a) gene on chromosome 6.^{4,5} The high variable number of KRINGLE 4 type2 domains results in an apo (a) size ranging from 187 kDa to over 662 kDa.

Assays with antibodies directed against this variable part of the Lp(a) molecule will underestimate Lp(a) in patients with apo (a) smaller than in the used calibrator and overestimate Lp(a) in such samples with larger apo (a) particles as in the calibrator. Based on the size heterogeneity it makes no sense to measure Lp(a) mass. Therefore the values should be expressed in terms of nanomoles per liter of Lp(a) protein.

Only standardization of these assays against an apo (a) size independent method will yield correct results. Such methods use antibodies that recognize a single copy of apo (a) per particle. By using the WHO/IFCC International Reference Reagent (SRM2B) this goal can be reached.⁶ The value in this material has been assigned by using two different ELISA's based on monoclonal antibodies specific to two different unique epitopes present in apo (a).^{7,8} High lipoprotein (a) concentrations in serum correlate with premature manifestation of atherosclerosis and strokes. When lipoprotein (a) concentrations exceed 75 nmol/L, the coronary risk is approximately doubled. In combination with elevated LDL-cholesterol concentrations, the risk increases approximately 6-fold. An elevated lipoprotein (a) level is considered to be the most sensitive parameter for the development of coronary heart disease, irrespective of other plasma lipoproteins. Lipoprotein (a) should be determined together with total cholesterol, HDL-cholesterol and LDL-cholesterol as well as triglycerides when assessing the total atherosclerotic risk. According to the European Atherosclerosis Society Lp(a) measurement should be recommended in selected cases at high risk and in subjects with a family history of premature cardiovascular disease.⁹

Test principle

Particle-enhanced immunoturbidimetric assay¹⁰

Human lipoprotein (a) agglutinates with latex particles coated with anti-Lp(a) antibodies. The precipitate is determined turbidimetrically at 659 nm.

Reagents - working solutions

R1 Glycine buffer: 170 mmol/L, pH 7.0; BSA; rabbit serum 0.1 %; stabilizers; preservative

SR Latex particles coated with polyclonal anti-human lipoprotein (a) antibodies (rabbit); glycine buffer: 170 mmol/L, pH 7.3; BSA; preservative

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.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

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 6 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 6 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 or K₂-EDTA and K₃-EDTA 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.

With K₃-EDTA tubes pay particular attention that the tubes are adequately filled.

Centrifuge samples containing precipitates before performing the assay.

Samples and controls are automatically prediluted 1:11 (1 + 10) with NaCl solution by the instrument.

Stability:

If samples are not assayed within 8 hours, samples should be stored at 2-8 °C.¹¹ If samples are not assayed within 48 h,¹¹ samples should be stored frozen at -70 °C or below.^{12,13} Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350 322, system-ID 07 5635 0 for automatic postdilution and standard serial dilutions. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.

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/plasma**COBAS INTEGRA 400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	D-R1-S-SR
Reaction direction	Increase
Wavelength A/B	659 nm
Calc. first/last	36-53
Typical prozone effect	> 450 nmol/L
Antigen excess check	No
Predilution factor	11
Unit	nmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	133 µL	
SR	33 µL	5 µL
Sample	20 µL	
Total volume	191 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	D-R1-S-SR
Reaction direction	Increase
Wavelength A/B	659 nm
Calc. first/last	48-78
Typical prozone effect	> 450 nmol/L
Antigen excess check	No
Predilution factor	11
Unit	nmol/L

Pipetting parameters

		Diluent (H ₂ O)
R1	133 µL	
SR	33 µL	5 µL
Sample	20 µL	
Total volume	191 µL	

Calibration

Calibrator	Preciset Lp(a) Gen. 2
	Use deionized water as zero calibrator.
Calibration mode	Spline
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Calibrators must be placed from the highest concentration first, to the lowest last, on the CAL/QC rack. 0 nmol/L calibrator is not provided with Preciset Lp(a) Gen.2. Please use deionized water as zero calibrator.

Traceability: This method has been standardized against the IFCC reference material SRM2B for nmol/L.¹⁴

Quality control

Quality control	PreciControl Lp(a) Gen. 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 concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: nmol/L × 0.4167 = mg/dL¹⁵

Limitations - interference

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

Icterus:¹⁶ 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:¹⁶ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):¹⁶ No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference up to a rheumatoid factors level of 1200 IU/mL.

Plasminogen: No significant cross reactivity in the tested concentration range (up to 150 mg/dL).

Apolipoprotein B: No significant cross reactivity in the tested concentration range (up to 200 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{17, 18}

High dose hook-effect: No false result occurs up to a lipoprotein (a) concentration of 450 nmol/L.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.¹⁹

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**

7-240 nmol/L

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 6 nmol/L
Limit of Detection	= 7 nmol/L
Limit of Quantitation	= 20 nmol/L

The Limit of Blank, the Limit of Detection and the 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 $n \geq 60$ 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 total error of 30 %. It has been determined using low concentration Lp(a) samples.

Expected values

A Lp(a) concentration of 30 mg/dL corresponding to the 75th percentile in a male Caucasian reference population is widely used as cut-off point or threshold value.^{20,21}

The European Atherosclerosis Society recommends screening for elevated Lp(a) in those at intermediate or high CVD/CHD risk and defines a desirable Lp(a) level ≤ 50 mg/dL.²²

However the NHLBI recommends to stop using data for total Lp(a) mass, and to use nmol/L units instead, which consider the number of particles. Additionally they recommend to use assays independent from apo(a) size and standardized according to the IFCC reference material SRM2B.²³

Based on the evaluation of Framingham data values above 75 nmol/L are regarded as a cut-off value for the presence of an increased risk.²³ Elevated Lp(a) levels can be found in most racial/ethnicity groups, with the prevalence being lowest in whites and Asians. The median Lp(a) levels in black subjects and in Asian Indians from southern locations are 2- to 4-fold higher compared with whites, and up to 68 % of blacks have Lp(a) levels > 75 nmol/L, whereas levels above this threshold are present in around 25 % of whites.²⁴ Therefore reference ranges have not been established for this assay for different ethnic populations or disease states. Since Lp(a) levels are largely influenced by hereditary factors and vary with ethnic populations it is recommended that each laboratory establish own expected values.

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. 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 nmol/L	SD nmol/L	CV %
PreciControl Level L	37.0	0.5	1.3
PreciControl Level H	136	1	0.6
Human serum 1	16.7	0.6	3.7
Human serum 3	86.3	0.5	0.6
Human serum 5	205	1	0.4

Intermediate precision	Mean nmol/L	SD nmol/L	CV %
PreciControl Level L	37.0	0.5	1.4
PreciControl Level H	136	1	0.7
Human serum 1	16.7	0.6	3.8
Human serum 3	86.3	1.0	1.1
Human serum 5	205	1	0.6

Method comparison

Lipoprotein (a) values for human serum and plasma samples obtained on a COBAS INTEGRA 800 analyzer (y) were compared with those determined using the corresponding reagent on a cobas c 501 analyzer (x).

Sample size (n) = 240

Passing/Bablok ²⁵	Linear regression
$y = 1.02x + 0.290$ nmol/L	$y = 1.01x + 0.972$ nmol/L
$r = 0.938$	$r = 0.999$

The sample concentrations were between 7.11 and 234 nmol/L.

Lipoprotein (a) values for human serum and plasma samples obtained on a COBAS INTEGRA 800 analyzer (y) were compared with those determined using the Northwest Lipid Metabolism and Diabetes Research Laboratories ELISA method traceable to the WHO/IFCC reference material SRM2B (x).

Sample size (n) = 105

Passing/Bablok ²⁵	Linear regression
$y = 1.01x + 1.92$ nmol/L	$y = 0.975x + 3.13$ nmol/L
$r = 0.939$	$r = 0.993$

The sample concentrations were between 7.10 and 218 nmol/L.

References

- Siekmeier R, Scharnagl H, Kostner GM, et al. Lipoprotein(a) - Structure, Epidemiology, Function and Diagnostics of a Cardiovascular Risk Marker. The Open Clin Chem J 2008;1:79-91.
- Kamstrup PR. Lipoprotein(a) and Ischemic Heart Disease- A Causal Association? A review: Atherosclerosis 2010 Jul;211(1):15-23.
- Genser B, Dias KC, Siekmeier R, et al. Lipoprotein(a) and Risk of Cardiovascular Disease - A Systematic Review and Meta Analysis of Prospective Studies. Clin Lab 2011;57(3-4):143-156.
- Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, et al. Genetically Elevated Lipoprotein(a) and Increased Risk of Myocardial Infarction. JAMA 2009;301(22):2331-2339.
- Clarke R, Peden JF, Hopewell JC, et al. Genetic Variants Associated with Lp(a) Lipoprotein Level and Coronary Disease. N Engl J Med 2009 Dec;361(26):2518-2528.
- Dati F, Tate JR, Marcovina SM, et al. First WHO/IFCC International reference Reagent for Lipoprotein(a) for immunoassay - Lp(a) SRM2B. Clin Chem Lab Med 2004;42(6):670-676.
- Marcovina SM, Albers JJ, Gabel B, et al. Effect of the Number of Apolipoprotein (a) Kringle 4 Domains on Immunochemical Measurements of Lipoprotein(a). Clin Chem 1995 Feb;41(2):246-255.
- Marcovina SM, Albers JJ, Wijsman E, et al. Differences in Lp(a) Concentrations and Apo(a) Polymorphs Between Black and White Americans. J Lipid Res 1996 Dec;37(12):2569-2585.
- Reiner Ž, Catapano AL, De Backer G, et al. ESC/EAS Guidelines for the management of dyslipidaemias. Eur Heart J 2011;32:1769-1818.
- Simó JM, Camps J, Gómez F, et al. Evaluation of a Fully Automated Particle-enhanced Turbidimetric Immunoassay for the Measurement of Plasma Lipoprotein(a). Population-Based Reference Values in an Area with Low Incidence of Cardiovascular Disease. Clin Biochem 2003 Mar;36(2):129-134.
- National Committee for Clinical Laboratory Standards, Procedures for the handling and Processing of Blood Specimens, Approved Guideline, NCCLS publication H18-A, Villanova, 1990.


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- 12 Simó JM, Camps J, Vilella E, et al. Instability of Lipoprotein (a) in Plasma Stored at -70 °C: Effects of Concentration, Apolipoprotein (a) Genotype, and Donor Cardiovascular Disease. Clin Chem 2001 Sep;47(9):1673-1678.
- 13 Sgoutas DS, Tuten T. Effect of Freezing and Thawing of Serum on the Immunoassay of Lipoprotein(a). Clin Chem 1992;38(9):1873-1877.
- 14 Marcovina SM, Albers JJ, Scanu AM, et al. Use of a reference Material Proposed by the International federation of Clinical Chemistry and Laboratory Medicine to Evaluate Analytical methods for the Determination of Plasma Lipoprotein (a). Clin Chem 2000 Dec;46(12):1956-1967.
- 15 Nordestgaard B, Chapman J, Ginsberg H. A Handbook for Clinicians, Lipoprotein (a): EAS Recommendations for Screening, Desirable Levels and Management. Sherborne Gibbs Ltd UK; 2012.
- 16 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
- 17 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
- 18 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.
- 19 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
- 20 Marcovina SM, Koschinsky ML. A Critical Evaluation of the Role of Lp(a) in Cardiovascular Disease: Can Lp(a) Be Useful in Risk Assessment? Semin Vasc Med 2002 Aug;2(3):335-344.
- 21 Shai I, Rimm EB, Hankinson SE, et al. Lipoprotein (a) and Coronary Heart Disease Among Women: Beyond a Cholesterol Carrier? Eur Heart J 2005;26:1633-1639.
- 22 Nordestgaard BG, Chapman MJ, Ray K, et al. Lipoprotein (a) as a cardiovascular risk factor: current status. Eur Heart J 2010 Dec;31(23):2844-2853.
- 23 Marcovina SM, Koschinsky ML, Albers JJ, et al. Report of the National Heart, Lung, and Blood Institute Workshop on Lipoprotein (a) and Cardiovascular Disease: Recent Advances and Future Directions. Clin Chem 2003 Nov;49(11):1785-1796.
- 24 Tsimikas S, Clopton P, Brilakis ES, et al. Relationship of Oxidized Phospholipids on Apolipoprotein B-100 Particles to Race/Ethnicity, Apolipoprotein (a) Isoform Size, and Cardiovascular Risk Factors: Results From the Dallas Heart Study, Circulation 2009 Apr;119(13):1711-1719.
- 25 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.

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

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