SDLDL

small dense LDL Seiken

Denka

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08834083190	small dense LDL Seiken (100 tests)	System-ID 21 5600 1	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

08834091190	small dense LDL-Calibrator (5 × 1 mL)	Code 20749	
08834105190	small dense LDL-Control Set small dense LDL Control Level 1 (5 × 1 mL) small dense LDL Control Level 2 (5 × 1 mL)	 Code 20348 Code 20349	

English

Roche does not hold the product registration for Partner Channels. The legal manufacturer indicated on the kit is solely responsible for all of the design, legal, and regulatory aspects of the product.

System information

SDLDL: ACN 21560

Intended use

In vitro test for the quantitative determination of small dense LDL cholesterol in human serum and plasma.

The small dense LDL Seiken test is used in conjunction with other lipid measurements and clinical evaluations to aid in the risk management of lipoprotein disorders associated with cardiovascular disease.

Summary

Elevated low-density lipoprotein cholesterol (LDL-C) in blood has long been regarded as a risk factor for coronary heart disease (CHD). Recently, LDL sub-fractions (subclasses) have emerged to further elucidate the association between lipid levels and development of CHD.[1,2,3,4] Small dense LDL (sdLDL) is one of such sub-fractions marked by smaller particle size and higher density. sdLDL is an atherogenic lipoprotein due to its higher ability to bind to the arterial wall and to penetrate into it, its lower binding affinity for the LDL receptor, its prolonged plasma half-life and its lower resistance to oxidative stress compared to that of large buoyant LDL (L LDL).[5,6,7,8] Various epidemiological and pathological studies have demonstrated the relationship between sdLDL-C level and CHD.[9,10] To date, ultracentrifugation and electrophoresis-based methods are used for the measurement of sdLDL-C but these methods are both laborious and time-consuming.[11] The SDLDL test is a direct method for the quantitative determination of sdLDL-C.

Test principle

The SDLDL test system is a multi-step method. sdLDL-C is measured on automated chemistry analyzers. The assay consists of two steps and is based on the technique of using well-characterized surfactants and enzymes that selectively react with certain groups of lipoproteins.

In the first step, non-sdLDL lipoproteins, that is, chylomicrons, VLDL, IDL, L LDL and HDL are decomposed by a surfactant and sphingomyelinase in reagent R1 that is reactive to those non-sdLDL lipoproteins. The cholesterol released from such non-sdLDL lipoproteins is then degraded to water and oxygen by the action of enzymes. Cholesterol ester is hydrolyzed by the cholesterol esterase (CHE) and then oxidized by the cholesterol oxidase (CO). Produced hydrogen peroxides are finally decomposed to water and oxygen by the catalase.

In the second step, another surfactant in reagent R3 releases cholesterol only from sdLDL particles and cholesterol released from sdLDL is then subject to the enzymatic reactions. As catalase in the reaction mixture is inhibited by sodium azide in reagent R3, hydrogen peroxides, produced from the reaction with the cholesterol esterase and cholesterol oxidase, then develop a purple-red color with the coupler in the presence of peroxidase (POD).

Step 1:

Chylomicrons-C, VLDL-C, IDL-C, L LDL-C and HDL-C $\xrightarrow{\text{CHE \& CO}}$ Cholestenone + fatty acid + H_2O_2

$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2\ H_2O + O_2$

Step 2:

sdLDL-C $\xrightarrow{\text{CHE \& CO}}$ Cholestenone + fatty acid + H_2O_2

$2\ H_2O_2$ + 4-AAP[a)] + TOOS[b)] $\xrightarrow{\text{POD}}$ purple-red color + $4\ H_2O$

a) 4-aminoantipyrine

b) N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

Reagents - working solutions

R1 Good's buffer, pH 7; cholesterol esterase (microorganism): < 58.33 µkat/L (3500 U/L); cholesterol oxidase (microorganism): < 33.33 µkat/L (2000 U/L); sphingomyelinase (microorganism): < 116.7 µkat/L (7000 U/L); catalase (microorganism): < 41.7 kµkat/L (2500 KU/L); N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS): < 5.0 mmol/L; surfactants; preservatives

R3 Good's buffer, pH 7; peroxidase (horseradish): < 200 µkat/L (12000 U/L); 4-aminoantipyrine: < 10.0 mmol/L; sodium azide: 0.05 %; surfactants; preservatives

R1 is in position B and R3 is in position C.

Note: Do not use if there is a crack or liquid leakage on the **cobas c** pack.

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: Human specimens and reaction waste shall be handled 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:

Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3 : 1).

Warning

H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P261	Avoid breathing mist or vapours.
P273	Avoid release to the environment.
P280	Wear protective gloves.

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Response:

P333 + P313	If skin irritation or rash occurs: 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

- Due to the circadian rhythm of sdLDL-C serum concentrations, it is recommended that specimens be collected in the morning.[12] Fasting samples are preferred.
- It is recommended that blood samples be processed to serum/plasma within 4 hours after collection; otherwise, samples should be refrigerated for up to 8 hours.
- Separate the serum/plasma from the clot or cells promptly.
- Refrigerate the samples after the separation.

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 and K_2-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.

Centrifuge samples containing precipitates before performing the assay.

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

Sample stability (serum/plasma)

It is recommended that samples be stored at refrigerated temperatures (2-8 °C) after the separation process. They may remain refrigerated for up to 3 days. For longer storage, samples should be stored frozen at –80 °C or below. At –80 °C samples are stable for 3 months.

If samples need to be shipped, they should be shipped at refrigerated conditions. For frozen samples, they should be shipped on dry ice.

Avoid subjecting samples to more than three freeze-thaw cycles.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

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.

The performance data below were generated using the **cobas c** 503. The **cobas c** 503 performance has been validated as comparable to **cobas c** 501. Similar performance has been demonstrated on other instruments.

Application for serum and plasma

Test definition

Assay type	2-Point End		
Reporting time	10 min		
Wavelength (sub/main)	700/600 nm		
Reaction direction	Increase		
Units	mmol/L (mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	85 µL	–	
R3	28 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent* (H_2O)
Normal	1.7 µL	–	–
Decreased	1.7 µL	–	–
Increased	1.7 µL	–	–

Calibration

Calibrators	S1: H_2O S2: small dense LDL Calibrator
Calibration mode	Linear
Calibration frequency	Full calibration • after 2 weeks • 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 against an in-house ultracentrifugation method.

The s LDL-EX "SEIKEN" assay was compared to the ultracentrifugation method using the Roche/Hitachi 917. The results obtained by the two methods were evaluated following the CLSI EP09c approved guideline.

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. 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 concentration of each sample.

Conversion factors:	mmol/L x 38.66 = mg/dL
	mg/dL x 0.0259 = mmol/L

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Limitations – interference

Concentrations of the potential interferents and the testing protocol were largely based on guidance CLSI Protocol EP07-A2.

Criterion: Recovery within ± 0.078 mmol/L (± 3 mg/dL) of initial values of samples < 0.776 mmol/L (30 mg/dL) and within ± 10 % for samples ≥ 0.776 mmol/L (30 mg/dL).

Icterus:[13] No significant interference up to an I index of 40 for conjugated bilirubin and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 684 µmol/L or 40 mg/dL; approximate unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[13] No significant interference up to an H index of 700 (approximate hemoglobin concentration: 109 µmol/L or 700 mg/dL).

Lipemia:[13] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Triglycerides: No significant interference from native triglycerides up to a concentration of 16.9 mmol/L (1500 mg/dL). This result was established and confirmed with a high triglyceride chylomicron fraction spiking test on a Roche/Hitachi 917 analyzer at Denka (formerly Denka-Seiken). The upper limit of the triglyceride tolerance may be lower depending on the individual sample.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

Sodium L-ascorbate: No significant interference from Sodium L-ascorbate up to a concentration of 5.048 mmol/L (100 mg/dL).

Uric acid: No significant interference from uric acid up to a concentration of 0.892 mmol/L (15 mg/dL).

Chyle: No significant interference from Chyle up to a concentration of 1420 FTU.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

The assessment of coronary heart disease (CHD) risk should include the patient's history, clinical information, and other clinical laboratory test results in addition to the results from this assay.

The sdLDL Seiken test is not a replacement for LDL-C measurement. It should not be used in risk assessment calculators.

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

0.103-2.587 mmol/L (4.0-100 mg/dL)

Lower limits of measurement

Limit of Blank, Limit of Detection, and Limit of Quantitation

Limit of Blank	= 0.008 mmol/L (0.3 mg/dL)
Limit of Detection	= 0.016 mmol/L (0.6 mg/dL)
Limit of Quantitation	= 0.041 mmol/L (1.6 mg/dL)

These data were generated on **cobas c** 501 at Denka, based on the Clinical and Laboratory Standards Institute (CLSI) Protocol EP17-A2. LoQ was lower than the lower measurement limit of the s LDL-EX "SEIKEN" kit (0.103 mmol/L (4.0 mg/dL)). The precision goal for the Limit of Quantitation was a CV % of less than 10 %.

Expected values

The data shown below has been obtained by the assay using s LDL-EX "SEIKEN". All studies (analytical and clinical) were performed on a Roche/Hitachi 917 analyzer. Similar performance has been demonstrated on other instruments.

Reference intervals

A reference interval study was performed in accordance with Clinical Laboratory Standard Institute (CLSI) protocol EP28-A3c. Eligible subjects were enrolled at two US regions and consented to a single blood draw after an overnight fast. Subjects were partitioned by age and gender, according to the following four parameters: (1) males 21-44 years, (2) males 45-75 years, (3) women 21-54 years (presumed pre-menopausal/peri-menopausal), and (4) women 55-75 years (presumed post-menopausal). The inclusion criteria for the reference populations were ambulatory status and presumptively healthy, HDL-C ≥ 1.035 mmol/L (40 mg/dL), LDL-C < 4.139 mmol/L (160 mg/dL), triglyceride < 2.258 mmol/L (200 mg/dL), fasting glucose < 6.993 mmol/L (126 mg/dL).

Age differences associated with the sdLDL-C level were significant in both genders (p = 0.0030 in males and p < 0.0001 in females). No significant difference was observed in the sdLDL-C level between males and females (p = 0.7564).

According to the CLSI guideline, the normal range was defined as the 2.5th percentile value to the 97.5th percentile value, as described below.

Group	Subjects of the study	Reference intervals
Younger group	Males 21-44 yrs and Females 21-54 yrs (n = 240)	0.329-1.249 mmol/L 12.7-48.3 mg/dL
Older group	Males 45-75 yrs and Females 55-75 yrs (n = 202)	0.326-1.337 mmol/L 12.6-51.7 mg/dL

Establishment with Multi-Ethnic Study of Atherosclerosis (MESA)

The Adult Treatment Panel of the National Cholesterol Education Program has generally selected the 75th percentile value for LDL cholesterol as being associated with high risk of CHD.[17] Based on this principle, the 75th percentile in normolipidemic and dislipidemic subjects who showed no signs of CHD or diabetes mellitus at baseline (n = 3938) was chosen. Using the MESA population, the analysis showed an sdLDL-C of 1.252 mmol/L (48.4 mg/dL), and was rounded to 1.293 mmol/L (50.0 mg/dL) as a cut-off value. Information about MESA is also available at (www.mesa-nhlbi.org).

Specific performance data

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

Precision

Precision testing was performed in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5-A3 requirements using 2 controls and 5 samples (2 aliquots per run, 2 runs per day, 22 days, for a total of 88 results per sample). The following results were obtained:

Repeatability	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Lipid control I	0.519 (20.1)	0.00648 (0.25)	1.3
Lipid control II	1.67 (64.6)	0.0186 (0.72)	1.1
Pool serum 1	0.166 (6.4)	0.00406 (0.16)	2.5
Pool serum 2	1.30 (50.3)	0.0192 (0.74)	1.5
Pool serum 3	2.37 (91.6)	0.0321 (1.24)	1.4
Pool serum 4	1.55 (59.9)	0.0162 (0.63)	1.0
Pool serum 5	0.789 (30.5)	0.0145 (0.56)	1.8

Intermediate precision	*Mean* *mmol/L (mg/dL)*	*SD* *mmol/L (mg/dL)*	*CV* *%*
Lipid control I	0.519 (20.1)	0.0102 (0.39)	2.0
Lipid control II	1.67 (64.6)	0.0285 (1.10)	1.7
Pool serum 1	0.166 (6.4)	0.00575 (0.22)	3.5
Pool serum 2	1.30 (50.3)	0.0248 (0.96)	1.9
Pool serum 3	2.37 (91.6)	0.0448 (1.73)	1.9
Pool serum 4	1.55 (59.9)	0.0324 (1.25)	2.1
Pool serum 5	0.789 (30.5)	0.0197 (0.76)	2.5

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

Method comparison

sdLDL cholesterol values for human serum 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) = 70

Passing/Bablok[18]	Linear regression
y = 1.025x – 0.008 mmol/L	y = 1.029x – 0.007 mmol/L
τ = 0.975	r = 0.999

The sample concentrations were between 0.120 and 2.36 mmol/L (4.64 and 91.2 mg/dL).

References

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2 Ai M, Otokozawa S, Asztalos BF, et al. Small dense LDL cholesterol and coronary heart disease: results from the Framingham Offspring Study. Clin Chem 2010 Jun;56(6):967-976.

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17 Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001 May 16;285(19):2486-2497.

18 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
GTIN	Global Trade Item Number
	Temperature limitation
IVD	In vitro diagnostic medical device
REF	Catalogue number
LOT	Batch code
	Use by date
	Store upright
UDI	Unique Device Identifier
	Importer
	Distributor
	Call
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123 IVD

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