BECKMAN COULTER

AU/DxC AU US

Instructions For Use

LDL
LDL-CHOLESTEROL

REF OSR6196 4 x 30 mL R1, 4 x 10 mL R2
OSR6296 4 x 50 mL R1, 4 x 16.5 mL R2

For *in vitro* diagnostic use only.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of LDL-Cholesterol concentrations in human serum and plasma on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

LDL-Cholesterol plays a causal role in the development of coronary heart disease (CHD). In 1988 the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) developed recommendations for the diagnosis and treatment of patients with hypercholesterolemia.[1] These recommendations defined LDL-Cholesterol as the primary target of therapy.

The 2001 update of these guidelines (NCEP-ATP III)[2] put further emphasis on better risk identification and more aggressive cholesterol-lowering treatment.

The guidelines classify LDL - Cholesterol levels as follows:

1. < 100 mg/dL	Optimal
2. 100 – 129 mg/dL	Near optimal/above optimal
3. 131 – 159 mg/dL	Borderline high
4. 160 – 189 mg/dL	High
5. ≥ 190 mg/dL	Very High

METHODOLOGY

The LDL-Cholesterol test is a two reagent homogenous system. The assay is comprised of two distinct phases. In phase one a unique detergent solubilizes cholesterol from non-LDL- lipoprotein particles. This cholesterol is consumed by cholesterol esterase, cholesterol oxidase, peroxidase and 4- aminoantipyrine to generate a colorless end product.

In phase two a second detergent in reagent 2 releases cholesterol from the LDL – lipoproteins. This cholesterol reacts with cholesterol esterase, cholesterol oxidase and a chromogen system to yield a blue color complex which can be measured bichromatically at 540/660nm. The resulting increase in absorbance is directly proportional to the LDL-C concentration in the sample.

Reaction phase 1

HDL-C, VLDL-C, LDL-C Chylomicrons $\xrightarrow{\text{CHE and CHO}}$ Cholest-4-en-3-one + Fatty acids + H_2O_2

H_2O_2 – 4-AAP $\xrightarrow{\text{Peroxidase}}$ LDL-C + Colorless end product

Reaction phase 2

LDL-C $\xrightarrow{\text{CHE and CHO}}$ Cholest-4-en-3-one + Fatty acids + H_2O_2

H_2O_2 + DSBmT+ 4-AAP $\xrightarrow{\text{Peroxidase}}$ Blue color complex

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Use fresh sample for analysis when possible. If analysis is delayed specimens are stable for 7 days when stored at 4 – 8°C. If specimens need to be stored for more than 7 days they may be preserved at less than - 20°C for up to 3 months.[3] Samples should only be frozen once.

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Serum, K2/K3 EDTA, or Li/Na heparinized plasma samples are the recommended specimens. Separate serum and plasma from red blood cells as soon as possible (within 3 hours). Plasma using anticoagulants such as citrate and oxalate are not recommended.[4]

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

LDL-Cholesterol Reagent

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.
3. This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

MES Buffer (pH 6.3)	
Cholesterol esterase (Pseudomonas)	1875 U/L
Cholesterol oxidase (Nocardia)	1125 U/L
Peroxidase (Horseradish)	975 U/L
Detergent 1	0.75 %
Detergent 2	0.25 %
DSBmT	0.25 mmol/L
4-aminoantipyrine	0.375 mmol/L
Ascorbate Oxidase	2250 U/L
Preservative	

GHS HAZARD CLASSIFICATION

LDL Cholesterol R1	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
LDL Cholesterol R2	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

LDL-Cholesterol Calibrator (Cat # ODC0024)

Storage location of the Calibrator in this laboratory:

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The LDL - Cholesterol reagent is ready for use. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagent is stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.
3. Do not use reagents that have been frozen.
4. Protect the reagents from direct sunlight.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, turbidity, or precipitate in the LDL - Cholesterol reagent may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

The frequency of calibration is every 7 days. Calibration of this LDL-Cholesterol procedure is accomplished by the use of the LDL-Cholesterol Calibrator material (Cat. # ODC0024).

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is mg/dL, for conversion to SI units (mmol/L) the result is divided by 38.7.

REPORTING RESULTS

EXPECTED RESULTS

National Cholesterol Education Program (NCEP) guidelines[2]

< 100 mg/dL	Optimal
100 - 129 mg/dL	Near Optimal / Above Optimal
130 - 159 mg/dL	Borderline High
160 - 189 mg/dL	High
≥ 190 mg/dL	Very High

Reference Intervals shown above were taken from the literature. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own

population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS (mg/dL)

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of studies[5] show that the following substances interfere with this LDL-Cholesterol procedure.

Ascorbate:	Interference less than 10% up to 20 mg/dL Ascorbate
Bilirubin:	Interference less than 10% up to 40 mg/dL conjugated Bilirubin
	Interference less than 10% up to 40 mg/dL unconjugated Bilirubin
Hemolysis:	Interference less than 10% up to 500 mg/dL Hemolysate
Lipemia:	Interference less than 10% up to 900 mg/dL Intralipid*
Globulin:	Interference less than 10% up to 5 g/dL added Gamma Globulin
Triglyceride:	Interference less than 10% or 10 mg/dL up to 750 mg/dL Triglyceride

*Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances is available.[6]

In very rare cases gammopathy, especially monoclonal IgM (Waldeström's macroglobulinemia), may cause unreliable results.

Laboratory specific procedure notes:

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The LDL-Cholesterol procedure is linear from 7.0 mg/dL to 400.0 mg/dL. Samples exceeding the upper limit of linearity should be manually diluted, using 1-part sample and 1-part physiological saline, repeated and the result multiplied by two. A concentration result is obtained from manually diluted samples in the > 400 mg/dL - 800 mg/dL range. Dilution of samples above 800 mg/dL is not supported. Samples can be repeated utilizing the AUTO REPEAT RUN.

SENSITIVITY

Typical change in absorbance for 1 mg/dL of LDL-Cholesterol is 1.8 mA.

Note:

Carry over from this LDL-Cholesterol reagent to Lipase reagent may result in elevated lipase values. Please refer to the User Update “Special Parameters – HDL/LDL Cholesterol Carryover Prevention” for proper programming instructions for your AU system.

METHODS COMPARISON

Reference[7]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 500 AU
X Method	DxC 700 AU
Slope	0.97
Intercept	2.164
Correlation Coeff. (r)	0.9992
No. of Samples (n)	132
Range (mg/dL)	7.770 - 367.200

PRECISION

Reference[7]

Estimates of precision, based on CLSI recommendations,[8] are consistent with typical performance. The within run precision is less than 3% CV and total precision is less than 5% CV. Assays of control sera were carried out and data reduced following CLSI guidelines.

N = 80	Within-run		Total	
Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
50.27	0.24	0.5	0.65	1.3
98.46	0.50	0.5	2.42	2.5
203.77	1.21	0.6	3.79	1.9

ADDITIONAL INFORMATION

DxC 700 AU analyzers require that each reagent application has a standard format of abbreviated Test Name. This Test Name is required to allow automated loading of the calibrator information for each application. Refer to the table below for the Test Name assigned to each application for this assay.

Test Name	Description
LDL1U	LDL-Cholesterol (Serum)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

Lot or Lot + Bottle

† Beckman Coulter LDL Cholesterol Calibrator Cat. No.: ODC0024

* Values set for working in mg/dL. To work in SI units (mmol/L) divide by 38.7

REVISION HISTORY

Updated Specimen Section

Updated REPORTING RESULTS section

Updated PROCEDURAL NOTES section

Updated Performance Characteristics section

Updated References section

Preceding version revision history

Add DxC 500i instrument to IFU

REFERENCES

1. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood cholesterol in Adults. The expert Panel. Arch Intern Med. 1988; 148: 36 - 69.

2. 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: 285: 2486 - 97.

3. Ehret W, Heil W, Schmitt Y, Töpfer G, Wisser H, Zawta B, et al. Use of anticoagulants in diagnostic laboratory investigations and stability of blood, plasma and serum samples. WHO/DIL/LAB/99.1

4. Tietz, Textbook of Clinical Chemistry, 3rd Edition, W.B. Saunders, 1999, 849.

5. CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

6. AACC Effects on Clinical Laboratory Tests: Drugs, Disease, Herbs and Natural Products https://clinfx.wiley.com/aaccweb/aacc/

7. Data is on file for specific AU/DxC AU analyzers.

8. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

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