LDL-CHOLESTEROL

OSR6196	**4 x 30 mL**	**R1**
	4 x 10 mL	**R2**
OSR6296	**4 x 50 mL**	**R1**
	4 x 16.5 mL	**R2**

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

Summary
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

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400 Beckman Coulter Analyzers**.**

Reagents
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		

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.

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

LDL-Cholesterol

Storage and Stability
1. The unopened reagents are 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.

Specimen Collection and Preparation
Serum, EDTA, or 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.[3]

Sample Storage and Stability
Use fresh sample for analysis when possible. If analysis is delayed specimens are stable for 5 days when stored at 2 – 8°C. If specimens need to be stored for more than 5 days they may be preserved at less than - 70°C for up to 3 months. Samples should only be frozen once.[4]

Interfering Substances
Results of studies[5] show that the following substances interfere with this LDL-Cholesterol procedure.
The criteria for no significant interference is recovery within 10% of the initial value.

Ascorbate:	No significant interference up to 20 mg/dL Ascorbate
Bilirubin:	No significant interference up to 40 mg/dL conjugated Bilirubin
	No significant interference up to 40 mg/dL unconjugated Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 900 mg/dL Intralipid*
Globulin:	No significant interference up to 5 g/dL added Gamma Globulin
Triglyceride:	No significant interference up to 1500 mg/dl Triglyceride**

* Intralipid,® manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.
** Triglyceride concentrate, manufactured by Miles Pentex, cat. no. 96-051-6, was used to measure triglyceride interference.

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. For further information on interfering substances, refer to Young[6] for a compilation of reported interferences with this test.

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

Procedure
A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
LDL-Cholesterol Reagent

Materials Required But Not Provided
LDL-Cholesterol Calibrator (Cat # ODC0024)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
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 analyzer, at least two levels of appropriate lipid control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, blanking, with each new lot of reagent, and after specific maintenance or trouble shooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
No manual calculations are required. Results are printed out automatically for each sample in mg/dL at 37°C. For S.I. Units (mmol/L), the results must be divided by 38.7.

Dynamic 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 diluted with physiological saline and repeated. The sample may be diluted, repeated, and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN. Samples with triglyceride values up to 1,293 mg/dL do not interfere with the results of the LDL-Cholesterol assay however samples with triglyceride levels greater than this should not be diluted.

Expected Values
Adults:[7] 75 – 193 mg/dL

Expected values may vary with age, sex, diet, and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics
The following data was obtained using the LDL-Cholesterol Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[9]

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 = 60	Within run		Total	
Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
52.91	0.41	0.77	1.12	2.12
98.97	0.56	0.57	2.27	2.30
125.08	0.92	0.74	2.85	2.28

Method Comparison[9]

Patient samples were used to compare this LDL-Cholesterol Reagent. The table below demonstrates representative performance on AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	0.96
Intercept	- 8.1
Correlation Coeff. (r)	0.965
No. of Samples (n)	115
Range (mg/dL)	16 – 188

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.

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. Tietz, Textbook of Clinical Chemistry, 3rd Edition, W.B. Saunders, 1999, 849.
4. Esteban-Salan, M, et al; Clin. Chem. 2000; 46:8, 1121 – 1131.
5. CLSI/NCCLS, Interference Testing in Clinical Chemistry EP7-P, 1986.
6. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
7. Beckman Coulter Inc. data on samples collected from 120 blood donors in Texas.
8. CLSI/NCCLS Evaluation Protocol EP5-T2, 1989.
9. Data is on file for specific AU analyzers.

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