DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
A – 2351 Wiener Neudorf, Austria, IZ-NÖ Süd, Hondastrasse, Objekt M55
Phone: ++43 (0) 2236 660910-0, Fax: ++43 (0) 2236 660910-30, e-mail: office@dialab.at

Liquid Reagents – ready to use

CHOLESTEROL HDL DIRECT

IMMUNOINHIBITION
2 Reagents

Diagnostic reagent for quantitative in vitro determination of high density lipoprotein cholesterol (HDL-C) in human serum or plasma on photometric systems.

REF	Kit Size	Content
F03220B	**1 x 12.5 L**	1 x 10 L R1 + 1 x 2.5 L R2
F03120B	**1 x 1.25 L**	1 x 1 L R1 + 1 x 250 mL R2
F03100	**5 x 100 mL**	4 x 100 mL R1 +1 x 100 mL R2
F03115	**5 x 50 mL**	4 x 50 mL R1 +1 x 50 mL R2
F03116	**5 x 25 mL**	4 x 25 mLR1 + 1 x 25 mL R2
F03117	**5 x 10 mL**	4 x 10 mL R1 + 1 x 10 mL R2
F16911	**5 x 50 mL**	4 x 50 mL R1 + 2 x 25 mL R2
F0416917	**5 x 62.5 mL**	1 x 62.5 mL R1 + 1 x 62.5 mL R2
FA0815	**5 x 50 mL**	5 x 40 mL R1 + 5 x 10 mL R2
FT1015	**4 x 62.5 mL**	4 x 50 mL R1 + 4 x 12.5 mL R2
FK0715	**5 x 50 mL**	4 x 50 mL R1 + 1 x 50 mL R2
FB0915	**2 x 150 mL**	2 x 120 mL R1 + 2 x 30 mL R2

Additionally offered:

F03710SV	1 x 3 mL	HDL-Cholesterol Calibrator	
D13585SV	1 x 2 mL	Lipid Calibrator	Diacal Lipids
D99486	3 x 3 mL	Lipid Control normal	Diacon Lipids
D99486SV	1 x 3 mL	Lipid Control normal	Diacon Lipids
D11487	3 x 3 mL	Lipid Control abnormal	Diacon Lipids High
D11487SV	1 x 3 mL	Lipid Control abnormal	Diacon Lipids High
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P

TEST PARAMETERS

Method:	Colorimetric, endpoint, increasing reaction, immunoinhibition
Wavelength:	600 / 700 nm (bichromatic)
Temperature:	37 °C
Sample:	Serum, heparinized plasma
Linearity:	up to 180 mg/dL (4.66 mmol/L)
Sensitivity:	The lower limit of detection is 1 mg/dL (0.03 mmol/L)

SUMMARY [1, 2]

Cholesterol is transported in plasma via lipoproteints, namely complexes between lipids and apolipoproteins. There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis. HDL-cholesterol has a protective effect impending plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-cholesterol values constitute an independent risk factor.

TEST PRINCIPLE

Dialab Cholesterol HDL Direct is a homogeneous method for HDL-cholesterol measurement without centrifugation steps. Antibodies against human lipoproteins form antigen-antibody complexes with LDL, VLDL and chylomicrons in a way that only HDL-cholesterol is selectively determined by an enzymatic cholesterol measurement [4].

LDL, VLDL, Chylomicrons $\xrightarrow{\text{Anti-human ß-lipoprotein antibodies}}$ Antigen-antibody complexes + HDL

HDL-Cholesterol + H_2O + O_2 $\xrightarrow{\text{CHE \& CHO}}$ Cholesten-3-on + fatty acid + H_2O_2

H_2O_2 + F-DAOS + 4-Aminoantipyrine $\xrightarrow{\text{POD}}$ blue colored complex + H_2O

ABBREVIATIONS

F-DAOS	=	N-Ethyl-N-(2-Hydroxy-3-sulfopropyl)-3,5-Dimethoxy-4-Flouroaniline, Sodium salt
CHE	=	Cholesterol Esterase
CHO	=	Cholesterol Oxidase
POD	=	Peroxidase

REAGENT COMPOSITION

COMPONENTS		CONCENTRATION	
Reagent 1			
Good´s Buffer	pH 7.0	25	mmol/L
4-Aminoantipyrine		0.75	mmol/L
Peroxidase		2000	U/L
Ascorbate Oxidase		2250	U/L
Anti human β-lipoprotein Ab. (sheep)			
Reagent 2			
Good´s Buffer	pH 7.0	30	mmol/L
Cholesterol Esterase		4000	U/L
Cholesterol Oxidase		20000	U/L
F-DAOS		0.8	mmol/L

REAGENT PREPARATION

Substrate Start:
Reagents are ready for use.
Sample Start:
Not possible (elimination of Non HDL-Chol. Lipoprotein fractions in first incubation step with Reagent 1).

REAGENT STABILITY AND STORAGE

Conditions:	Protect from light Close immediately after use Do not freeze the reagents! Avoid contamination.
Storage:	at 2 – 8 °C
Stability:	up to the indicated expiration date

NOTE: It has to be mentioned, that the measurement is not influenced by occasionally occurring colour changes, as long as the absorbance of the premixed reagent (4 parts R1 + 1 part R2) is < 0.03 at 600 – 700 nm.

SAMPLE STABILITY AND STORAGE [5]

Stability:	at 20 – 25 °C	2 days
	at 4 – 8 °C	7 days
	at - 20 °C	3 months

Discard contaminated specimens. Freeze only once!

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

	Blank	Sample/Cal.
Sample/ Calibrator	---	10 µL
Reagent 1	1000 µL	1000 µL
Mix. Incubate for 5 min. at 37°C. read absorbance (A1), then add:		
Reagent 2	250 µL	250 µL
Mix, incubate for 5 min. at 37°C, read absorbance (A2). ΔA = [(A2-A1) sample or calibrator] - [(A2-A1) blank]		

CALCULATION

$$\text{HDL [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}} \times \text{Conc. Calibrator [mg/dL]}$$

UNIT CONVERSION

mg/dL x 0.02586 = mmol/ L

REFERENCE RANGE [7] *

≥ 35 mg/dL (0.9 mmol/L)

* Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges as necessary.

Rev 08, 2018-05-23

DIALAB Produktion und Vertrieb von chemisch – technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
A – 2351 Wiener Neudorf, Austria, IZ-NÖ Süd, Hondastrasse, Objekt M55
Phone: ++43 (0) 2236 660910-0, Fax: ++43 (0) 2236 660910-30, e-mail: office@dialab.at

Clinical Interpretation
Epidemiological studies have observed that low HDL-cholesterol concentrations < 39 mg/dL (0.9 mmol/L) in men and < 43 mg/dL in women, especially if associated with fasting triglycerides > 180 mg/dL (2 mmol/L), predict a high risk of coronary heart disease [2]

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE
The test has been developed to determine HDL Cholesterol concentrations within a measuring range from 1 –180 mg/dL (0.03 – 4.66 mmol/L). If concentration exceeds 180 mg/dL, samples should be diluted 1 + 2 with NaCl (9 g/L sodium chloride in water) and results multiplied by 3.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 1 mg/dL (0.03 mmol/L).

PRECISION

Intra-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	24.0	0.31	1.27
Sample 2	49.0	0.26	0.52
Sample 3	97.7	0.64	0.65

Inter-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	27.3	0.54	2.00
Sample 2	58.0	0.57	0.98
Sample 3	98.6	1.34	1.36

SPECIFICITY/INTERFERENCES
no interference up to:

Ascorbic acid	50 mg/dL
Bilirubin	50 mg/dL
Bilirubin conjugated	40 mg/dL
hemoglobin	500 mg/dL
triglycerides	1200 mg/dL

For further information on interfering substances refer to Young DS [6].

METHOD COMPARISON
A comparison of Dialab HDL Cholesterol (y) with a commercially available test (x) using 100 samples gave following results:
y = 1.05 x + 0.571 mg/dL; r = 0.995.

CALIBRATION
The assay requires the use of a HDL Cholesterol Calibrator.
We recommend the Dialab **HDL-Cholesterol Calibrator** or the lipid calibration plasma **Diacal Lipids**.
The value in the the HDL-Cholesterol Calibrator is traceable to the CDC reference method Ultracentrifugation/Heparin-Mn, and in Diacal Lipids to NIST SRM® 1951 Level 2.

QUALITY CONTROL
All control sera with HDL Cholesterol values determined by this method can be used.
We recommend the Dialab lipid control sera **Diacon Lipids** and **Diacon Lipids High** and the Dialab multi control sera **Diacon N** (with values in the normal range) and **Diacon P** (with values in the pathological range).
Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION
Special applications for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS
1. Reagent 1: Warning
   H317: May cause an allergic skin reaction.
   P280: Wear protective gloves/protective clothing/eye protection/face protection.
   P302+P352: IF ON SKIN: Wash with plenty of water/soap.
   P333+P313: If skin irritation or rash occurs. Get medical advice/attention.
2. In very rare cases, samples of patients with gammopathy might give falsified results [8].
3. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
4. When using enzymatic methods for the determination of cholesterol esters, contamination and interference to other clinical chemistry assays on the same instrument in principle cannot be exluded. In the event of such a problem occurring, please refer to the instrument'smanual for channel setting and washing procedure options.
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only!

WASTE MANAGEMENT
Please refer to local legal requirements

REFERENCES
1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. P. 809-61.
2. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. Eur Heart J 1998; 19: 1434-503.
3. Wiebe DA, Warnick GR. Measurement of high-density lipoprotein cholesterol. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington. AACC Press, 1997. p. 127-44.
4. Nauck M, Maerz W, Wieland H. New immunoseparation-based homogenous assay for HDL-cholesterol compared with three homogenous and two heterogeneous methods for HDL-cholesterol ClinChem 1998; 44: 1443-51.
5. Guder WG, Zawta B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p.22-3.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
7. Schaefer EJ, McNamara J. Overview of the diagnosis and treatment of lipid disorders. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press; 1997. p. 25-48.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: Mechanism, detection and prevention. Clin Chem lab Med 2007; 45(9); 1240-1243.

CE 2°C 8°C IVD

Rev 08, 2018-05-23