HDL-Cholesterol (HDL-C)

(Direct Enzymatic Method)

- **Kit Specifications:**

	Reagent/Quantity	Storage
Cat. No.: **HD0211**	R1. 1 x 45 ml R2. 1 x 15 ml total 60 ml	2-8°C
Cat. No.: **HD0011**	R1. 3 x 40 ml R2. 2 x 20 ml total 160 ml	2-8°C
Cat. No.: **HD0111**	R1. 6 x 40 ml R2. 2 x 40 ml total 320 ml	2-8°C
Cat. No.: **HD0217**	R1. 1 x 45 ml R2.1 x 15 ml total 60 ml	2-8°C
Cat. No.: **HD0017**	R1. 2 x 60 ml R2. 2 x 20 ml total 120 ml	2-8°C
Cat. No.: **HD0117**	R1.4 x 60 ml R2.2 x 40 ml total 320 ml	2-8°C

- **Intended Use:**

In Vitro Diagnostic reagent pack for the quantitative determination of High Density Lipoprotein Cholesterol (HDL-C) in human serum and plasma on automated and semi-automated photometric systems.

- **Summary and Explanation:**

Cholesterol, synthesized by body cells and absorbed with food, is a component of cell membranes, a precursor for steroid hormones and bile acids. Cholesterol is transported in plasma via lipoproteins, complexes between lipids and Apo lipoproteins. Four lipoprotein classes exist: High density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. These classes show distinct relationship to coronary atherosclerosis. LDL is involved in the cholesterol transport to the peripheral cells, contributing to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. HDL-C has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-C values constitute an independent risk factor. One of the important functions of HDL involves the physiological removal of cholesterol from peripheral tissues and cells, and transport to the liver. The concept that HDL could protect against CHD primarily originated from epidemiological studies of the healthy population, in particular the Framingham study. In addition, a number of antioxidant effects, HDL also serves as a powerful mediator of the cellular inflammatory and antithrombotic responses. HDL-particles are macromolecule complexes synthesized by liver and intestine and formed from surface components. HDL-particles are released into plasma during lipolysis of lipoproteins rich in triglycerides. Particles consist of an amphipathic lipid monolayer of phospholipids and cholesterol with embedded amphipathic proteins surrounding a core of hydrophobic lipids, mostly cholesteryl esters and triglycerides. HDL-C monitoring is highly relevant in cardiovascular risk assessment. Elevated HDL-C levels usually correlate with decreased cardiovascular risk; whereas reduced concentrations of HDL-C, especially in combination with elevated triglycerides are associated with high risk of atherosclerotic heart disease, even at or below recommended LDL-C goals. Preferred screening tests for dyslipidemia or lipid disorders are total cholesterol (TC) and HDL-C but the majority of screening guidelines nowadays recommend a full lipid profile including TC, LDL-C, HDL-C and triglycerides. Selective chemical precipitation techniques are widely used for the determination of HDL-C such as heparin-manganese, dextran-magnesium and phosphotungstate-magnesium. However, these techniques require physical separation via centrifugation, which is not suited to large scale laboratory use. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

- **Principle of the Method:**

The direct HDL Cholesterol assay is a homogeneous method for directly measuring serum HDL-C levels without the need for any pre-treatment and centrifugation steps. First step, the substances with high affinity to block LDL, VLDL and chylomicrons to involving in this enzyme reaction.

Second step, special surfactant that selectively accelerates reaction with the enzyme reagent with HDL cholesterol and determining them.by the color pigmentation. The base on measurement at (578-620) 600 Nano meter. The intensity of the color is related to the amount of HDL on the sample.

$$\text{LDL/VLDL/Chylomicrons} \xrightarrow[\text{CHE+CO}]{} \text{non-reactive LDL/VLDL/Chylomicrons}$$

$$\text{HDL + Cholesterol + O2} \xrightarrow[\text{POD}]{} \text{Cholestenone + H2O2}$$

$$\text{H2O2 + 4-Aminoantipyrine + HSDA} \longrightarrow \text{Colored Compound}$$

- **Reagent Preparation and Stability:**

Reagent is ready for use.
Before use, mix reagent by gently inverting each bottle.
Reagent is stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
Do not use reagent over the expiration date.
Do not freeze and protect from light.
R1: transparent and colorless.
R2: transparent and light yellow.

- **Waste Management:**

Refer to local legal requirements for chemical disposal regulations.
Warning: Handle waste as potentially biohazardous material.
Dispose of waste according to accepted laboratory instructions and procedures.

- **Warnings and Precautions:**

For In Vitro Diagnostics Use Only.
For Professional Use Only.
Carefully read instructions for use.
In case of serious damage to the bottle or cap, resulting in product leakage or contamination, do not use the reagent pack and contact your distributor.
Take all necessary precautions required when handling laboratory reagents.
Do not use components past the expiry date stated on the Bottles.
Do not interchange caps among components as contamination may occur and compromise test results. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.

- **Type of Specimen:**

Use fresh and non-hemolysis serum or heparinized plasma as specimen.
Do not use EDTA plasma.
Specimen must be completely cleared before assay.
It is recommended to follow CLSI procedures (or similar standardized conditions) regarding specimen handling. Specimen should be collected in an appropriate sample container, with proper specimen identification. The stability of the samples on 4 days at 2-8°C and -20°C for 14 days. The serum/plasma should be collected immediately after blood collection. Avoid any contamination, freezing and DE freezing the samples.

- **Materials Required but not Supplied:**

Direct HDL/LDL Cholesterol Calibrator from TKS or other valid calibrators.
Lipid control Level 1 & 2 from TKS or other valid controls.
Saline solution 0.9 % NaCl
General laboratory equipments

- **Notes:**

Carefully read instructions for use.
It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with hydrochloric acid 1 N and then thoroughly rinsed it with distilled water.
Use clean disposable pipette tips for its dispensation.
Disposable and glassware material used must be free of metals, ions and detergents.

- **Performance Characteristics:**

Performance results can vary with the instrument used.
Data obtained in each individual laboratory may differ from these values.

Maximum determination in this assay 120 mg/dl.
For samples with a higher concentration (120 mg/dl), dilute 1:1 with 0.9 % NaCl and re-assay. Multiply result by 2.
LOD: The lowest detectable level was estimated at 2 mg/dl.

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Precision:

Intra assay-Within run HDL-C

Sample	n	Mean (mg/dl)	SD (mg/dl)	CV (%)
1	20	51	0.23	0.45
2	20	95.3	1.24	1.3

Inter assay-Between run HDL-C

Sample	n	Mean (mg/dl)	SD (mg/dl)	CV (%)
1	20	50	1.47	2.94
2	20	93.4	3.68	3.94

- **Accuracy:**

Results obtained using BIOMEDIC reagents (y) did not show systematic differences when compared with other commercial reagents (x).
Correlation coefficient (r): 0.9869
Regression equation: Y = 1.119 (X) + 0.0428 mg/dl
The results of the performance characteristics depend on the analyzer used.

- **Interfering Substances:**

No interferences were observed to:

Ascorbic acid	Less than 5% interference up to 50 mg/dl
Haemolysis	Less than 5% interference up to 500 mg/dl
Bilirubin	Less than 5% interference up to 20 mg/dl
Intralipid	Less than 5% interference up to 500 mg/dl

- **Reference Values:**

Age	Male mmol/L	Male mg/dl	Female mmol/L	Female mg/dl
<60 years	**0.78-1.63**	**30-63**	**0.85-2.25**	**33-87**
>60 years	**0.78-1.94**	**30-75**	**0.85-2.49**	**33-96**

Each laboratory should establish its own expected values. The HDL results should always be reviewed with the patient's medical examination and history.

- **Assay Procedure:**

Allow reagents to reach working temperature before using.
A proportional variation of the reaction volumes indicated does not change the result.

Assay conditions:

Wave length	**600 nm**
Temperature	**37°C**
Cuvette	**1 cm**

Adjust the instrument to zero with distilled water.

Control/Sample/Calibrator	Blank	
900 µl	**900 µl**	**R1**
12 µl	-	**Control/Sample/Calibrator**
Gently mix and incubate at 37°C **for 5 minutes**. Measure the first absorbance A1 and A calibrator against the blank.		

300 µl	300 µl	R2
Gently mix and incubate 37°C for 5 minutes. Measure the second sample A2 and calibrator against the blank. **ΔA=A2 – A1**		

Calculation:

HDL-C (mg/dl) = ΔA Sample X Conc. Cal (mg/dl)
ΔA Calibrator

- **Conversion Factor:**

HDL-C (mg/dl) x 0.02586 = HDL-C (mmol/L)

TOSE'E KIMIA SA'ADAT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

- **References:**

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4-Tietz N., Fundamentals of clinical chemistry Philadelphia W.B. Saunders 335-337, 1976.
5-Young D.S., Effect of Drugs on Clinical Laboratory Tests, AACC 5thed.2000
6-Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th edition (2018).
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9-Third report of the National Cholesterol Education Programmed (NCEP) Expert Panel on detection, Evaluation and treatment of High blood cholesterol in adults (Adult treatment Panel III). JAMA Publication, Vol 285, No.19, P2486-2497; 2001.
10-Shih WJ, Bachorik PS, Haga JA, Myers GL, Stein EA; Clinical Chemistry, 2000; 46:3:351-364.

- **Symbols:**

The following symbols are used in the labelling of TOSE'E KIMIA SA'ADAT systems:

IVD	In Vitro Diagnostics	Σ	Contains sufficient for <n> tests
LOT	Batch Code		Temperature limit
REF	Catalogue No.		Consult instruction for use
	Expiry Date		Caution
	Date of Manufacture		Keep dry
	Manufactured by		This way up
	Biological Risks		Keep away from sunlight

ISO 13485:2016
ISO 9001:2015

IVD

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