Cholesterol (CHO)
(CHOD/PAP Method)

- **Kit Specifications:**

	Reagent/Quantity	Storage
Cat. No.: **CH0011**	R.1: 6 x 50 ml total 3·0 ml	2-8°C
Cat. No.: **CH0017**	R.1: 5 x 60 ml total 300 ml	2-8°C

- **Intended Use:**
In Vitro Diagnostic reagent pack for the quantitative determination of Cholesterol in human serum/plasma on automated and semi-automated photometric systems.

- **Summary and Explanation:**
Cholesterol is an essential of cell membranes, for preparing of steroids hormones and bile acids which synthesis in the cell. Also, absorb it from the foods. Cholesterol in plasma transport by lipoproteins which is the complex of lipids and Apolipo proteins. Lipoproteins categorized according the density. The low density of lipoprotein is LDL, very low lipoprotein is VLDL, high lipoproteins HDL and chylomicrons. LDL roles is to transfer cholesterol to inside tissue and HDL harvest cholesterol from tissue. In the study shows that close relation between high LDL in serum of the patient (heart and vascular coronary diseases) and any other of arteriosclerosis, even if they have a normal range of cholesterol. The LDL goes high so the high risk to susceptible to get above diseases. HDL against LDL play the healthy role to remove cholesterol from tissue so the high HDL low risk in heart and vascular disease. Meanwhile, the low of HDL in normal cholesterol make high risk on those diseases. So the measurement of cholesterol is essential to screening the heart vascular patients. Also, it is key point for estimate of heart attack time ratio of HDL and LDL is necessary.

Principle of the Method:
This method is based on hydrogen peroxide produced caused by hydrolyzed and oxidation of cholesterol. This reaction is done by addition of 4-amino antipyrine and phenol with peroxidase activity. The color of quinonimine complex which measurement on 500 to 550 nm. The intensity of the color will have measured on 500-550 Nano meter wavelength which is correlate with the amount of cholesterol in sample.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{4-Cholesterol-3-one} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{4-chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

- **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 reagents over the expiration date.
Do not freeze and protect from light.
The reagent is straw color and transparent.

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 serum, plasma heparinized with EDTA. Do not collect the plasma with oxalate, citrate or fluoride. The stability of Cholesterol in the samples at 2-8°C for 7 days and at-20°C for 3 months.
The serum / plasma should have collected during 2 hours after blood collection. Avoid any contamination.

Required but not Supplied:
General chemistry calibrator from TKS or other valid calibrators.
General chemistry 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 is 690mg/dl
LOD: 2 mg/dl
For samples with a higher concentration (690mg/dl), dilute 1:1 with 0.9 % NaCl and re-assay. Multiply result by 2.
Precision:
Intra Assay-Within run Cholesterol

Sample	n	Mean (mg/dl)	SD (mg/dl)	CV (%)
1	20	106.71	1.35	1.27
2	20	175.11	1.65	0.94
3	20	261.09	1.07	0.41

Inter Assay-Between run Cholesterol

Sample	n	Mean (mg/dl)	SD (mg/dl)	CV (%)
1	20	98.05	1.71	1.74
2	20	164.25	2.10	1.28
3	20	234.4	2.39	1.02

- **Accuracy:**
Results obtained using BIOMEDIC reagents (y) did not show systematic differences when compared with other commercial reagents (x).
Correlation coefficient (r): 0.999
Regression equation: Y = 0.997 (X) – 0.028 mg/dl
The results of the performance characteristics depend on the analyzer used.

- **Interfering Substances:**
the less concentration of below items are not interfering in this assay.

Bilirubin (mixed isomer)	Less than 10% interference up to 600 µmol/L Bilirubin
Lipaemia	Less than 10% interference up to 5 g/L Intralipid.
Haemolysis	Less than 10% interference up to 5g/l Hemoglobin.
Ascorbic Acid	5 mg/dl

- **Reference Values:**

Manufactured by: TOSE'E KIMIA SA'ADAT, No.5, 32nd Alley, Asadabadi St. Yousef abad, Tehran-IRAN
Factory Address: No.18, Niloufar 6, Toska Blvd., Nakhl Blvd., Paytakht Industrial Town, 54 km of Tehran-Semnan Road
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Serum/Plasma	
Optimal/Normal	**<200 mg/dl**
Borderline/Prognosis	**200-240 mg/dl**
High/Abnormal	**>240 mg/dl**

Each laboratory should establish its own expected values. The Cholesterol 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:

505nm	**Wavelengths**
30-37 °C	**Incubation Temperature**
1 cm	**Cuvette**

Adjust the instrument to zero with distilled water.

Control/Sample/Calibrator	**Blank**	
1000 µl	1000 µl	**R**
10 µl	-	**Control/Sample/Calibrator**
Gently mix and incubate for 10 minute at 37°C. Then measure the absorbance from A sample and A calibrator.		

- **Calculations:**

Cholesterol (mg/dl) = Abs. Sample x Cal/STD.Conc. (mg/dl)
Abs. STD/Cal

Conversion units:
Cholesterol (mg/dl) x 0.02586 = Cholesterol (mmol/L)

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

- **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 Compony; 1999.p.809-61.
2-Cole TG, Klotzsch SG, McNamara J. Measurement of triglyceride concentration. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997.p.115-26.
3-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.
4-Fossati P. Prencipe L., Clin.Chem., 28(1982)2077-80
5-NCCLS documents M29-T2.2nd Ed. 1991.
6-Klotzsch, S.G.&MC Namara, R.j.clin.chem.1190;36:1605-13.

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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