EN

CHOLESTEROL FOOD

REF 984324

❶ 3 x 16 ml Reagent

INTENDED USE
Reagent for photometric determination of Cholesterol in homogenous liquid samples using automated Thermo Scientific Arena or Gallery analyzer.

METHOD
Enzymatic test with Cholesterol oxidase (CHO), Peroxidase (POD) and with colorimetric indicator Chinonimin.
Method is performed at 37 ℃, using 540 nm filter.

PRINCIPLE OF THE PROCEDURE
Cholesterin + O_2 ---CHO---> Cholest-4-en-3-one + H_2O_2
$2H_2O_2$ + 4-Amoniantipyrin + Phenol ---POD---> Chinonimin + $4H_2O$

REAGENT INFORMATION
Reagent 3 x 16 ml

Note: Labels of reagents vials have two barcodes.
For Arena analyzers, turn the short barcode on the left side to the barcode reader.
For Gallery analyzers, turn the long barcode on the right side to the reading position of the reagent rack.

Concentrations

Good's Buffer	pH 6.7
Phenol	5 mmol/l
4-Amoniantipyrin	0.3 mmol/l
Cholesterol oxidase (CHO)	≥ 100 U/l
Peroxidase (POD)	≥ 3 U/l

Precautions
See the separate Safety Data Sheet for hazardous components.
The reagents contain sodium azide (< 0.1 %) as preservative. Do not swallow. Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

Preparation
The reagent is ready-to-use.
Note: Check that there are no bubbles on the surface of the reagent when you insert vials in the analyzer.

Storage and Stability
Reagents in unopened vials are stable at 2…8 ℃ until the expiry date printed on the label. Do not freeze the reagents.
Refer to the Application Notes of your analyzer for the on board stability of reagents.

SAMPLES

Sample Type
Food and other sample material.

Sample concentration and Arena/Gallery application
All method related details are in the separate application note.

Example application has no primary dilution. This means that automatic dilution is not in use.

Sample preparation for determination of free cholesterol
Free cholesterol is extracted from the sample with isopropanol. Homogenize approximately 10 g of e.g. sausage. Weight 1 g of homogenized sample and extract cholesterol with 3 x 5 ml of isopropanol. Filter solution into 25 ml volumetric flask, add 5 ml of 8 M HCl and fill up to the mark with isopropanol.,Let it stay at +4 ºC for 20 min in order to get the fatty layer separated. Filter and use the clear solution for the test.

Sample preparation for determination of total cholesterol
Esterified cholesterol is hydrolyzed under alkaline conditions and then extracted together with free cholesterol through the addition of isopropanol. Weight approximately 1 g of liquid egg or 2 g of mayonnaise into a 50 ml round-bottom flask with a 1 g of see-sand. Add 10 ml of fresh 1.0 M methanolic potassium hydroxide (Mix 10 M KOH and methanol 1+9) and 10 ml of isopropanol and heat and stirr for 30 min under a reflux. Allow to cool to room temperature and fill up to the mark with isopropanol. Filter turbid solution and use the clear solution for the test.

TEST PROCEDURE
See a separate application for the Arena or Gallery analyzer.

Materials required but not provided
Isopropanol (2-Propanol, C_3H_8O) and general laboratory equipment.

Cholesterol Food Std Cat no. 984391 is not included in the kit.

Calibration
Cholesterol Food Std can be used or other. Ordering code for Cholesterol Food Std is 984391 (3x3 ml). Because of the high concentration of the standard, it needs to be diluted manually before use with isopropanol 1+4. As a sample zero, 90 % isopropanol solution is used.

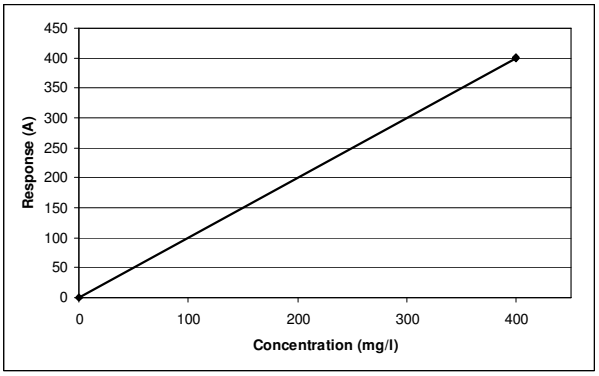
Quality Control
Use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

Available controls:
Cholesterol Food Std can be used. If Cholesterol Food Std is used also for calibration, an additional internal control is recommended to be used.

CALCULATION OF RESULTS
The results are calculated automatically by the analyzer using a calibration curve.

Conversion factors:
mg/l x 0.00259 = mmol/l
mmol/l x 386.65 = mg/l

Calibration Curve (example)



Calibrator	Response (A)	Calc. conc. (mg/l)
Isopropanol (90%)	0.005	0
Cholesterol Std	0.254	398.982

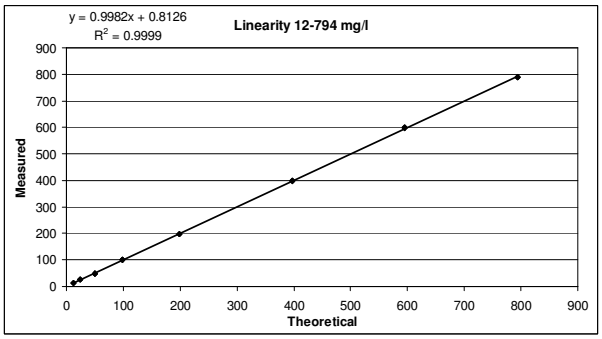
Calibration factor of this example is 1607.
Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

Interference
The determination is specific for Cholesterol. Other sterols are only slightly cross-reacting, because of the high specifity of the cholesterol oxidase.

MEASURING RANGE
The test has been developed to determine Cholesterol concentrations within a measuring range from 20 to 1000 mg/l.

PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given.



Determination limit (=Test limit low)
The determination limit is the lowest concentration that can be measured quantitatively. The determination limit for this method is 20 mg/l.

Precision

Arena analyzer

	Mean 172 mg/l		Mean 434 mg/l		Mean 746 mg/l	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.931	0.5	2.040	0.5	3.014	0.4
Between run	2.653	1.5	3.688	0.8	2.546	0.3
Total	2.812	1.6	4.215	1.0	3.946	0.5

A precision study was performed using Arena 20XT for 5 days, with the number of measurements being n = 50.

Gallery analyzer

	Mean 65 mg/l		Mean 350 mg/l		Mean 656 mg/l	
	SD	CV %	SD	CV %	SD	CV %
Within run	0.434	0.7	1.197	0.3	1.802	0.3
Between run	1.364	2.1	1.319	0.4	3.058	0.5
Total	1.431	2.2	1.781	0.5	3.549	0.5

A precision study was performed using Gallery for 5 days, with the number of measurements being n = 50.

WASTE MANAGEMENT
Please refer to local legal requirements. Empty the cuvette waste bin daily immediately after the analysis.

OTHER REMARKS
All results must be verified by laboratory quality control samples.
Manufacturer does not warrant that the product is error-free or will accomplish any particular result. In no event shall the manufacturer be liable for special, incidental, indirect, punitive or consequential damages (including, but not limited to, loss of profits, loss of goodwill, loss of data or loss of use damages) arising out of the use or disposition of the products.

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Changes from previous version
New insert.