# LIQUIZYME CHOLESTEROL

(CHOD / POD Method)

Code	Product Name Pack Size	
LS012B	Liquizyme Cholesterol	2 x 50 ml
LS012C	Liquizyme Cholesterol	6 x 50 ml
LS012G	Liquizyme Cholesterol	1 x 50 ml
LS012H	Liquizyme Cholesterol	10 x 50 ml
LS012I	Liquizyme Cholesterol	20 x 50 ml

#### Intended Use

Diagnostic reagent for quantitative in vitro determination of Cholesterol in human serum and plasma.

#### Clinical Significance

Measurement of serum cholesterol levels can serve as an indicator of liver function, biliary function, intestinal absorption, propensity towards coronary artery disease, thyroid function and adrenal disease. Cholesterol levels are important in the diagnosis and classification of hyperlipoproteinaenias. Stress, age, gender, hormonal balance and pregnancy affect normal cholesterol levels.

#### Principle

This reagent is based on the formulation of Allain et al and the modification of Roeschlau with further improvements to render the reagent stable in solution.

1. Cholesterol ester + 
$$H_2O$$
  $\longrightarrow$  Cholesterol + Fatty acids

CHO

2. Cholesterol +  $O_2$   $\longrightarrow$  Cholesterol +  $O_2$  POD

3.  $O_2$  + 4AAP + Phenol  $\longrightarrow$  Quinoneimine dye +  $O_2$ 

where:

CHE = Cholesterol Esterase
CHO = Cholesterol Oxidase
4AAP = 4-aminoantipyrine
POD = Peroxidase

- 1. Cholesterol esters are enzymatically hydrolysed by cholesterol esterase cholesterol and free fatty acids
- Free cholesterol, including that originally present, then oxidized by Cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.
- $3. The hydrogen peroxide combines with 4 aminoantipyrine \\ to from a chromophone (quinoneimine dye) which may \\ be quantitated at 505 nm.$



#### Reagent Composition

Reagent 1 : Cholesterol Enzyme Reagent
Goods Buffer : >80 mmol/L
Phenol : >10 mmol/L

 Phenol
 :
 >10 mmol/L

 4-aminoantipyrine
 :
 >0.3 mmol/L

 Cholesterol esterase
 :
 >250 U/L

 Cholesterol oxidase
 :
 >250 U/L

 Peroxidase
 :
 >2250 U/L

 Reagent 2: Cholesterol Standard
 :
 200 mg/dl

Ready to use

#### Reagent Preparation

Reagent is liquid, ready to use.

#### Stability And Storage

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at  $+2-+8^{\circ}C$ .

# Material Required But Not Provided

- Clean & Dry container.
- Laboratory Glass Pipettes or Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

#### Specimen Collection And Handling

Use serum, plasma (heparin, EDTA).

It is recommended to follow NCCLS procedures (or similar standardized conditions).

### Stability In Serum/Plasma:

7 days : at  $+2-+8^{\circ}$ C Discard contaminated specimens.

#### Calibration

Calibration with the Cholesterol standard provided in the kit is recommended.

#### **Quality Control**

It's recommended to run normal and abnormal control sera to validate reagent performance.

# **Unit Conversion**

ma/dl x 0.026 = mmol/L

#### **Expected Values**

Serum : 130 - 250 mg/dl

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

# Performance Data

Data contained within this section is representative of performance on Beacon system. Data obtained in your laboratory may differ from these values.

#### Precision

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Intra-assay precision	Mean	SD	CV		
Within run (n=20)	(mg/dl)	(mg/dl)	(%)		
Sample 1	158.70	2.00	1.26		
Sample 2	290.85	4.18	1.44		
Inter-assay precision	Mean	SD	CV		
Run to run (n=20)	(mg/dl)	(mg/dl)	(%)		
Sample 1	96.80	1.08	1.12		

#### Comparison

A comparison between Cholesterol (y) and a commercially available test (x) using 20 samples gave following results:

y = 0.9942 x + 1.5149 mg/dl

r = 0.999

#### Interferences

Following substances do not interfere:

haemoglobin up to 5 g/l, bilirubin up to 20mg/dl, triglycerides up to  $2000\,\text{mg/dl}.$ 

#### Warning And Precautions

For in vitro diagnostic use. To be handled by entitled and professionally educated person.

Reagents of the kit are not classified like dangerous but contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

#### Waste Management

Please refer to local legal requirements.

**Assay Procedure** 

Wavelength : 505 nm Cuvette : 1 cm

Addition Sequence	Reagent Blank	Standard	Sample
Reagent 1	1000 μΙ	1000 μΙ	1000 μΙ
Standard	-	10 μΙ	-
Sample	-	-	10 μl
Distilled Water	10 μΙ	-	-

Mix and incubate 5 min. at  $+37^{\circ}$ C. Measure absorbance of the sample Abs. T and standard Abs. S against reagent blank. The final color is stable for one hour.

#### Calculation

Cholesterol (mg/dl) =  $\frac{Abs. I}{}$  x 200 Abs. S

# Applications for automatic analysers are available on request.

## Assay Parameters For Photometers

Mode	End point
Wavelength 1 (nm)	505
Sample Volume (μl)	10
Reagent Volume (µl)	1000
Incubation time (min.)	5
Incubation temp. (°C)	37
Normal Low (mg/dl)	130
Normal High (mg/dl)	250
Linearity Low (mg/dl)	4.2
Linearity High (mg/dl)	1000
Standard Concentration	200 mg/dl
Blank with	Reagent
Unit	mg/dl

#### References

- Searcy, R. L. "Diagnostic Biochemistry" McGraw-Hill, New York, NY. 1996.
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#### Symbols Used On Labels

REF

Catalogue Number



Manufacturer

 $\Box i$ 

See Instruction for Use



Lot Number



Content



Storage Temperature



Expiry Date



In Vitro Diagnostics

