LIQUIZYME

TRIGLYCERIDES

(GPO/POD Method)

BEACON

Code	Product Name	Pack Size
LS027A	Liquizyme Triglycerides	4 x 50 ml
LS027D	Liquizyme Triglycerides	6 x 50 ml
LS027H	Liquizyme Triglycerides	1 x 50 ml
LS027I	Liquizyme Triglycerides	2 x 50 ml
LS027J	Liquizyme Triglycerides	10 x 50 ml
LS027K	Liquizyme Triglycerides	20 x 50 ml

Intended Use

Diagnostic reagent for quantitative in vitro determination of Triglycerides in human serum, plasma.

Clinical Significance

Triglycerides are a family of lipids absorbed from the diet and produced endogenously from carbohydrates. Measurement of triglycerides is important in the diagnosis and management of hyperlipidemias. These diseases can be genetic or secondary to other disorders including nephrosis, diabetes mellitus and endocrine disturbances. Elevation of triglycerides has been identified as a risk factor for atherosclerotic disease.

Principle

The series of reactions involved in the assay system is as follows;

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Free Fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{DHAP + } H_2O_2$$

$$H_2O_2 + \text{phenolic Chromogen} \xrightarrow{\text{POD}} \text{Red coloured Compound}$$

Triglycerides are enzymatically hydrolyzed by lipase to free acids and glycerol. The glycerol is phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate (ADP). GLycerol-3-phosphate is oxidized to dihydroxy-acetone phosphate (ADP) by glycerol phosphate oxidase producing hydrogen peroxide (H_2O_2). In a Trinder type color reaction catalyzed by peroxidase, the H_2O_2 reacts with 4-aminoantipyrine (4AAP)

Reagent Composition

Reagent 1 : Triglycerides Enzyme Reagent

Pipes buffer	:	>45 mmol/L
4-Chlorophenol	:	>3 mmol/L
ATP	:	>1.5 mmol/L
Glycerolkinase	:	<1000 U/L
Peroxidase	:	>2000 U/L
Lipoproteinlipase	:	>2500 U/L
Glycerol-3-phosphate-Oxidase	:	>1000 U/L
4-Aminoantipyrine	:	>0.25 mmol/L

Reagent 2 : Triglycerides Standard : 200 mg/dl

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°C.

Materials Required But Not Provided

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

Specimen Collection And Handling

Use unheamolyse serum, plasma (EDTA, Heparin).
It is recommended to follow NCCLS procedures (or similar standardized conditions).

Stability :

2 days	:	at +20 – +25°C
7 days	:	at +4 –+ 8°C

Calibration

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

Quality Control

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

Expected Values

Normal : 60 to 170 mg/dl

It is recommended that each laboratory verify this range or derive 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.

Limit of quantification : 4.00 mg/dl
Linearity : 1000 mg/dl
Measuring range : 4.00 – 1000 mg/dl

Precision

Intra-assay precision Within run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	90.95	1.54	1.69
Sample 2	256.95	4.50	1.75

Inter-assay precision Run to run (n=20)	Mean (mg/dl)	SD (mg/dl)	CV (%)
Sample 1	81.53	1.68	2.07

Comparison

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

y = 0.985 x + 1.420 mg/dl
r = 0.999

Interferences

Following substances do not interfere:
Haemoglobin upto 10 g/l, bilirubin up to 40 mg/dl.
Interference by N-acetylcysteine (NAC), acetoaminophen and metamizole causes falsely low results. To carry out the test, blood withdrawal should be performed prior to administration of drugs.

Warning And Precautions

For in vitro diagnostic use. To be handles by entitled and professionally educated person.
Reagents of the kit are not classified as dangerous.

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 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl
Distilled Water	10 µl	-	-

Mix and incubate 10 min. at +37°C. Measure absorbance of the sample Abs. T and standard Abs. S against reagent blank.

Calculation

$$\text{Triglycerides (mg/dl)} = \frac{\text{Abs. T}}{\text{Abs. S}} \times 200$$

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.)	10
Incubation temp. (°C)	37
Normal Low (mg/dl)	60
Normal High (mg/dl)	170
Linearity Low (mg/dl)	4
Linearity High (mg/dl)	1000
Standard Concentration	200 mg/dl
Blank with	Reagent
Unit	mg/dl

References

1. Rifai N, Bachorik PS, Alberts 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. 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. 155-26
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. Tietz Textbook of Clinical Chemistry and Molecular diagnostics. Burtis, C.A., Ashwood, E.R., Bruns, D.E.; 5th edition, WB Saunders Company, 2012

Symbols Used On Labels

REF	Catalogue Number		Manufacturer
	See Instruction for Use	LOT	Lot Number
CONT	Content		Storage Temperature
	Expiry Date	IVD	In Vitro Diagnostics

BEA/24/TRI/LS/IFU Ver-03
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