TRIGLYCERIDES-L (Single Liquid)

ENZOPAK

(GPO METHOD)

Last update 09-2020

Ref. CC3-TGS.18N, 5x25 ml
CC3-TGS.18NU, 5x60 ml

INTENDED USE

Reagent kit for quantitative estimation of Triglycerides in serum or plasma.

PRODUCT HIGHLIGHTS

- Lipase/GK/GPO-Reagent.
- Very sensitive chromogen.
- Internationally recommended standard giving accuracy as per International requirements.

INTRODUCTION

Conventional methods for the estimation of triglycerides have been chemical or enzymatic. In the enzymatic methods, triglycerides are hydrolysed to release glycerol by use of lipase. There are various enzymatic methods to estimate liberated glycerol.

Liquid Triglycerides is formulated using Lipo-Protein Lipase (LPL), Glycerokinase (GK), Glycerol-3-Phosphate Oxidase (GPO) and Peroxidase (POD) for quantitative estimation of serum triglycerides. High molar extinction coefficient of the final coloured complex makes the method quite sensitive.

DIAGNOSTIC SIGNIFICANCE

Normally, triglycerides, HDL-cholesterol, total cholesterol are estimated, and LDL-cholesterol is calculated. These parameters represent a routine practical aspect of lipid profile which is useful in determination of risk factor or health status of a subject.

Serum triglycerides estimation is an important parameter in the investigation of hyperlipoproteinaemia. Elevated levels may be found in atherosclerosis, diabetes mellitus, glycogen storage diseases like in Von Gierke's disease, secondary hyperlipoproteinaemia, alcoholism and nephrotic syndrome.

PRINCIPLE

Lipase hydrolyses triglycerides sequentially to Di & Monoglycerides and finally to glycerol. Glycerol Kinase (GK) using ATP as P04 source converts Glycerol liberated to Glycerol-3-Phosphate (G-3-Phosphate). G-3-Phosphate Oxidase (GPO) oxidises, G-3-Phosphate formed to Dihydroxy acetone phosphate and hydrogen peroxide is formed. Peroxidase (POD) uses the hydrogen peroxide formed, to oxidise 4-Aminoantipyrine and chlorophenol to a pink coloured complex. The absorbance of the coloured complex is measured at 520nm (500-550 nm or with green filter) which is proportional to Triglyceride concentration.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty Acids}$$

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

$$\text{Glycerol-3-Phosp.} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone Phosphate} + H_2O_2$$

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

PRESENTATION

All reagents to be stored at 2-8^{0}C	No. of Bottles 5x25 ml	5x60 ml
• Triglycerides-L (Single Liquid) Reagent	5	5
• Triglycerides Standard (200 mg/dl)	1	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Buffer	100 mmol/L
• LPL	$\geq$ 1000 U/L
• ATP	1mmol/L
• GK	$\geq$ 1000 U/L
• GPD	$\geq$ 2000 U/L
• 4-AAP	0.3 mmol/L
• Chlorophenol	1.2 mmol/L

pH 7.0 $\pm$ 0.1 at 25^{0}C
Triglycerides Standard (200 mg/dl)
Also contains non-reactive filters and Stabilizers.

PRECAUTION

Liquid Triglycerides is for *IN-VITRO* diagnostic use only.
Reagent contains Sodium Azide. DO NOT INGEST.

PREPARATION OF WORKING REAGENT

Triglycerides reagent (Liquid) is ready to use.

REAGENT STORAGE & STABILITY

Liquid Triglycerides reagent is stable at 2-8^{0}C until the expiry date indicated on the label (18 months). Protected from light.

SPECIMEN COLLECTION

Fresh, clear fasting serum with no hemolysis should be used. Heparin/ citrated plasma may be used. No other anticoagulant is suitable. Serum levels are slightly (5mg/dl) higher than plasma levels.

REACTION PARAMETERS

- Type of Reaction : End Point
- Wavelength : 520 nm (500-550 nm)
- Flowcell Temperature : 37^{0}C
- Incubation : 10 min. at 37^{0}C
- Std. Concentration : 200 mg/dl
- Sample Volume : 20 Microlitres (0.02 ml)
- Reagent Volume : 1.0 ml.
- Zero setting with : Reagent Blank
- Light Path : 1.0 cm.

TEST PROCEDURE

Pipette into Test Tubes	BLANK	STANDARD	TEST
Working Reagent (ml)	1.0	1.0	1.0
Standard (ml)	-	0.020	-
Sample (ml)	-	-	0.020

Mix and incubate at 37^{0}C for 10 minutes and read absorbance of test and standard against reagent blank at 520 nm (500-550 nm or Green filter).

TEST RESULTS

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std.}} \times 200$$

To convert (mg/dl) to mmol/lit use the following equation mmol/lit = mg/dl x 0.0114

NORMAL VALUES

Male : 65-190 mg/dl
Female : 45-170 mg/dl

LINEARITY

This method is linear upto 800 mg/dl. For sample values higher than 800 mg/dl, dilute the samples suitably with 0.9% saline and repeat the assay. Apply proper dilution factor to calculate the final results.

REFERENCES

1. FOSSATI P., LORENZO, P.,: Serum Triglycerides determined colorimeterically with an enzyme that produces hydrogen peroxide, Clin. Chem 28.2077 - 2080(1982).
2. McGOWAN, M. W. ARTISS, J. D. STRANBERG, D. R. ZAK, B. A.,: Peroxidase coupled method for the colorimetric determination of serum Triglycerides, Clin. Chem.29, 538-542 (1983)

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