TRIGLYCERIDES LIQUID

ENZOPAK

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(GPO METHOD)
Reagent kit for quantitative estimation of Triglycerides in serum or plasma.

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

BACKGROUND AND SYNOPSIS:
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.
ENZOPAK 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.

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.

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

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

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

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

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.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
* Buffer	100 mmol/L
* LPL	≥ 1000 U/L
* ATP	1mmol/L
* GK	≥ 1000 U/L
* GPD	≥ 2000 U/L
* 4-AAP	0.3 mmol/L
* Chlorophenol	1.2 mmol/L

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

CE

An ISO 13485: 2012 & GMP Certified Company

PRESENTATION:

	No. of Bottles			
All reagents to be stored at 2-8^{0}C	5x5 ml	5x20 ml	4x50 ml	5x100 ml
Liquid Triglycerides Reagent	5	5	4	5
Triglycerides Standard (200 mg/dl)	1	1	1	2

PRECAUTION:
ENZOPAK Liquid Triglycerides is for *IN-VITRO* diagnostic use only.
Regent contains Sodium Azide. DO NOT INGEST.

REAGENT STORAGE & STABILITY:
ENZOPAK 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.

GENERAL 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.

PROCEDURE:
For laboratories using instruments with 1.0 ml. / 0.5 ml. cuvette capacity.

PIPETTE INTO	Procedure for 1 ml.			Procedure for 0.5 ml.		
TEST TUBES	BLK	STD.	TEST	BLK	STD.	TEST
WORK. RGT. (ml)	1.0	1.0	1.0	0.5	0.5	0.5
STD. (ml)	-	0.020	-	-	0.010	-
SAMPLE (ml)	-	-	0.020	-	-	0.010

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).

PROCEDURE FOR COLORIMETERS (2.5 ml.)

PIPETTE INTO TEST TUBES	BLANK	STANDARD	TEST
WORKING REAGENT (ml)	1.0	1.0	1.0
STANDARD (ml)	-	0.05	-
SAMPLE (ml)	-	-	0.05

Mix well and incubate at 37^{0}C for 15 minutes.

Dist. Water (ml.)	1.5	1.5	1.5

Mix and read absorbance of test and standard against reagent blank at 520 nm (500-550 nm or Green filter).

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

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

NORMAL VALUES :
Serum Triglycerides
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 :

FOSSATI P., LORENZO, P.,: Serum Triglycerides determined colorimeterically with an enzyme that produces hydrogen peroxide, Clin. Chem 28.2077 - 2080(1982).

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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