TRIGLYCERIDES

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

- Lipase/GK/GPO-Reagent with 24 months expiry.
- Very sensitive chromogen.
- Internationally recommended standard giving accuracy of 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 Triglycerides is formulated using Lipoprotein 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 PO_4 source converts Glycerol liberated to Glycerol-3-Phosphate (G-3-Phosphate). G-3-Phosphate Oxidase (GPO) oxidises G-3-Phosphate & forms Dihydroxy acetone phosphate and hydrogen peroxide. Peroxidase (POD) uses the hydrogen peroxide formed, to oxidise 4-Aminoantipyrine and TOOS (N-ethyl-N-Sulphohydroxy propyl-m Toluidine) to a purple coloured complex. The absorbance of the coloured complex is measured at 546 nm (530-570 nm or with yellow filter) which is proportional to Triglyceride concentration.

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

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

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

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

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 Von Gierke's disease, secondary hyperlipoproteinaemia, alcoholism and nephrotic syndrome.

REAGENT COMPOSITION

Active Ingredients	**Concentration**
Reagent-1	
• LPL	$\geq$ 30000 U/L
• GK	$\geq$ 1500 U/L
• GPO	$\geq$ 3500 U/L
• POD	$\geq$ 2000 U/L
• 4 - AAP	20 mmol/L
• ATP	0.3 mmol/L
Reagent-2	
• Buffer	100 mmol/L
• TOOS	1 mmol/L

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

PRESENTATION :

	No. of Bottles
All reagents to be stored at 2-8^0C	15 x 1.1 ml.
1 Triglycerides (Enzymes, Chromogen)	15
2 Triglycerides (Buffer)	1
* Triglycerides Standard (200 mg/dL)	1

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

PREPARATION OF WORKING REAGENT :
FOR 15 X 1.1 ml.
Dissolve the contents of one Vial of 1 TRIGLYCERIDES with 1.1 ml. of 2 TRIGLYCERIDES (Buffer). Mix gently to dissolve.

REAGENT STORAGE & STABILITY :
ENZOPAK Triglycerides reagents are stable at 2-8^0 C until the expiry date indicated on the label.
Working reagent is stable for 2 weeks at 2-8^0 C, when stored in dark coloured container.

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

REACTION PARAMETERS :

- Type of Reaction : End Point
- Wavelength : 546 nm (530-570 nm)
- Flowcell Temperature : 37 ^{0}C
- Incubation : 15 min. at 37 ^{0}C
- Std. Concentration : 200 mg/dL
- Sample Volume : 10 µl (0.01 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 TEST TUBES	Procedure for 1 ml.			Procedure for 0.5 ml.		
	BLK	STD.	TEST	BLK	STD	TEST
• WORK. RGT. (ml)	1.0	1.0	1.0	0.5	0.5	0.5
• STD. (ml)	-	0.01	-	-	0.005	-
• SAMPLE (ml)	-	-	0.01	-	-	0.005

Mix and incubate at 37^0 C for 15 minutes and read absorbance of test and standard against reagent blank at 546 nm (530-570 nm or yellow filter).

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PROCEDURE FOR COLORIMETERS (2.5 ml.) :

PIPETTE INTO TEST TUBES	BLANK	STANDARD	TEST
• WORKING REAGENT (ml)	1.0	1.0	1.0
• STANDARD (ml)	-	0.02	-
• SAMPLE (ml)	-	-	0.02

Mix well and incubate at 37^{0}C for 20 minutes.

• Dist. Water (ml.)	1.5	1.5	1.5

Mix and read absorbance of test and standard against reagent blank with yellow 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 :

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 colorimetrically 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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