# TRIGLYCERIDES (DST)

**ENZOPAK**
Last update 04-2014

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

- **Lipase/GK/GPO-Reagent.**
- **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 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 $P0_4$ 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 DHBS (3,5, dichloro-2-hydroxy benzene sulphate) to a red coloured complex. The absorbance of the coloured complex is measured at 520 nm (500-550 nm) or with GREEN filter which is proportional to Triglyceride concentration.

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

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

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

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

**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 | ≥ 1000 U/L |
| GK | ≥ 800U/L |
| GPO | ≥ 1500 U/L |
| POD | ≥ 2500 U/L |
| 4 – AAP | 0.25 mmol/L |
| ATP | 1 mmol/L |
| **Reagent -2** | |
| Buffer | 100 mmol/L |
| DHBS | 0.5 mmol/L |
| pH 8.0 ± 0.5 at 25$^0$ C | |
| **Triglycerides Standard (200 mg/dl)** | |

Also contains non-reactive fillers and stabilizers.

**PRESENTATION**

| All reagents to be stored at 2-8$^0$C | No. of Bottles | | | |
|---|---|---|---|---|
| • 1 Triglycerides (Enzymes, Chromogen) | 5x10ml<br>5 | 5x20ml<br>5 | 4x50ml<br>4 | 20x50ml<br>20 |
| • 2 Triglycerides (Buffer) | 1 | 5 | 4 | 20 |
| • Triglycerides Standard (200 mg/dl) | 1 | 1 | 1 | 4 |

**For 5x10 ml reconstitution bottle provided**

**PRECAUTION**
ENZOPAK Triglycerides (DST) i s for *IN-VITRO* diagnostic use only.
**Reagent contains Sodium Azide. DO NOT INGEST.**

**PREPARATION OF WORKING**
**REAGENT FOR 5 x 10 ml.**
Carefully transfer the content of 1 Triglyceride (Powder) into the bottle containing 10 ml of 2 Triglyceride (Buffer). Mix well to dissolve Wait for 5-minutes before use.

**FOR 5 x 20 ml.**
Carefully transfer the content of 1 Triglyceride (Powder) into the bottle containing 20 ml of 2 Triglyceride (Buffer). Mix well to dissolve Wait for 5-minutes before use.

**FOR 4 x 50 ml. & 20 x 50 ml**
Carefully transfer the content of 1 Triglyceride (Powder) into the bottle containing 50 ml of 2 Triglyceride (Buffer). Mix well to dissolve Wait for 5-minutes before use.

**REAGENT STORAGE & STABILITY**
ENZOPAK Triglycerides (DST) reagents are stable at 2-8$^0$ C until the expiry date indicated on the label.

Working reagent is stable for 6 months at 2-8$^0$ C, when stored in original container protected from light.

**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 (5mg/dl) higher than plasma levels.

**GENERAL PARAMETERS**

| | | |
|---|---|---|
| • Type of Reaction | : | End Point |
| • Wavelength | : | 520 nm (500-550nm) |
| • Flowcell Temperature | : | 37$^0$ C |
| • Incubation | : | 5 min. at 37 $^0$C |
| • Std. Concentration | : | 200 mg/dl |
| • Sample Volume | : | 20 µl (0.020 ml) |
| • Reagent Volume | : | 1.0 ml. |
| • Zero setting with | : | Reagent Blank |
| • Light Path | : | 1.0 cm. |

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# TRIGLYCERIDES (DST)

**ENZOPAK**

**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 5 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 10 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).

**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 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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