Pariksha Biotech
A game changer in IVD

Triglycerides Estimation Kit

High-Q Triglycerides- ML

GPO - PAP Trinder's Method

HIGH®
Command on Quality

Intended use: Enzymatic in vitro test for the quantitative determination of triglycerides in human serum and plasma.

Summary & Clinical Significance:
Triglycerides are esters of the trihydric alcohol glycerol with 3 long chain fatty acids. They are partly synthesized in the liver and partly ingested in food. The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases. The enzymatictriglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase.

Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis.

This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for therapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazoneand ADPS (N-ethyl-N-(3-sulfopropyl) m-anisidine) under the catalytic action of peroxidase to form a blue purple complex .

Principle:

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

Glycerol + ATP $\xrightarrow{GK}$ Glycerol - 3 - Phosphate (G - 3 - P)

G - 3 - P + O_2 $\xrightarrow{GPO}$ H_2O_2 + Dihydroxyacetone phosphate

H_2O_2 + 4 - Aminoantipyrine + N-Ethyl-N-(3-sulfopropyl)-3-methoxyaniline $\xrightarrow{POD}$ Blue Purple Complex + H_2O + Hcl

The triglycerides present in the serum are catabolised into Glycerol and free fatty acids by Lipoprotein Lipase. Liberated Glycerol is converted to Glycerol-3-phosphate in presence of Glycerol Kinase and ATP. Glycerol 3 phosphate is acted upon by Glycerol-3-phosphate Oxidase to form Hydrogen Peroxide. This together with Phenolic compound ADPS and 4-Aminoantipyrine in presence of Peroxidase gives the blue purple colour complex. The intensity of the colour is measured at 546 nm (540-546 nm) and is proportional to the Triglycerides concentration in serum samples.

Normal Range:
Serum Triglycerides : Up to 150 mg/dl
It is recommended that laboratories should establish their own normal range.

Storage and Stability:
All the reagents must be stored at 2-8^{0}C and are stable till the expiry date mentioned on the labels.
When opened contamination must be avoided.

Specimen:
Serum/EDTA Plasma
Do not use hemolysed samples.

Active Ingredients

1. Lipase	- > 5 KU/L
2. Glycerol Kinase	- > 1.25 KU/L
3. Glycerol Phosphate Oxidase	- > 5 KU/L
4. Peroxidase	- > 2 K U/L
5. ATP	- > 2 m mol/L
6. 4AAP	- > 10 m mol/L
7. ADPS	- > 0. 2 m mol/L
8. Goods Buffer	- > 20 m mol/L
9. Surfactants and stabilizers	

Reagent Presentation:
The reagents included in the kit are ready to use.
Important Note: High-Q Triglycerides reagent incorporates an aggressive and superior chromogen ADPS which gives the faster reaction than the conventional chromogens . A slight blue purple colour up to 0.200 Abs may be seen in the reagent blank which does not affect the performance of the reagents.

Procedure:
1. Always use well washed soap or detergent free Glass Tubes for testing. Do not use recycled plastic tubes as they react with ADPS Chromogen leading to the false results.

Pipette into test tubes libeled Blank (B), Standard (S) and Test (T) as follows:

Reagent	B	S	T
Triglycerides Reagent	1.0 ml	1.0 ml	1.0 ml
Triglycerides Standard (Conc:200 mg/dl)	–	10 µl	–
Specimen	–	–	10 µl

Mix well incubate for 10 minutes at 37°C

Mix and read absorbance of Standard (S) and Test (T) against Reagent Blank (B) at 546 nm

Calculations:

$$\text{Triglycerides in mg/dl} = \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \times 200 \quad \text{(Standard Concentartion)}$$

Imprecision:
Reproducibility was determined using human samples the following results were obtained:

	within run		
Sample	Mean(mg/dl)	SD(mg/dl)	CV %
Control serum 1	122	1.09	0.89
Control serum 2	150	1.79	1.19
Control serum 3	206	1.44	0.70
	between day		
Control Serum	Mean(mg/dl)	SD(mg/dl)	CV %
Control serum 1	121	1.96	1.62
Control serum 2	161	1.84	1.14
Control serum 3	204	2.36	1.16

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System Parameters:

Reaction Mode	:	End Point
Units	:	mg/dl
Wave Length	:	546 nm (540-546)
Blanking with	:	Reagent
Flow Cell Temp.	:	37^{0}C
Low Normal	:	0
High Normal	:	150
Sample Volume	:	10 µl
Reagent Volume	:	1000 µl
Linearity	:	1200
Standard Conc.	:	200

Quality Control:
To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of Quality Control material checks both, the instrument and the reagent function.

Notes:
1.Do not use recycled plastic tubes as they react with ADPS Chromogen leading to the false results. Always use well washed soap or detergent free Glass Tubes

2.Contamination of Standard and Reagents must be avoided. After use all the reagents must be immediately stored back at 2-8^{0}C.

3.Replug the Triglycerides Standard vial after use. Use clean glassware & microtips while pipetting Triglycerides standard.

4.Contamination by soap or glycerol will affect this assay.

5.No interference was observed by ascorbic acid up to 32 mg/dl, bilirubin up to 43 mg/dl, hemoglobin up to 500 mg/dl and lipemia up to 2200 mg/dl triglycerides.

6.For sample values higher than 1200 mg/dl, dilute the sample with normal saline and multiply the result with appropriate dilution factor.

7.As with all the diagnostic procedures, the physician should evaluate the data obtained by use of this kit in light of other clinical information.

References:

1.Jacobe, N.J., Van Demark, P.J. (1960) Arch Biochem Biophys. 88, 250.
2.Trinder, P. (1960) Amn. Clin. Biochem., 6,24.
3.Bucolo G., David M. Clin. Chem 19,476 (1973).

Ordering Information

Cat No:	Pack Size	Presentation
P-TGL-50	2 x 25 ml	Mono Reagent
P-TGL-100	4 x 25 ml	
P-TGL-250	5 x 50 ml	

Product Features

- **Liquid Stable, Ready to use Mono Reagent**
- **Superior ADPS Chromogen**
- **10 Minutes End Point Reaction**
- **Lipid Clearing Factor (LCF)**
- **Aqueous Triglycerides standard provided (Standard Conc: 200 mg/dl)**
- **Linearity: 1200 mg/dl)**
- **Measuring Wavelength 546 nm (540 – 546 nm)**
- **Serum/ EDTA Plasma are the specimens**
- **Available as multipurpose reagents and dedicated system packs**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

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Manufactured in India by :
Pariksha Biotech Pvt Ltd,
Plot no.1/B-14, SVICE,
Balanagar,
Hyderabad-500037
Telangana State

+91 7075706709
info@parikshabio.com
www.parikshabio.com