TRIGLYCERIDES
GPO / PAP METHOD

Cat no.	Size
414001	4*25
414002	2*50
414003	4*50
414004	2*100

INTENDED USE
Quantitative determination of triglycerides in human serum and plasma.

DIAGNOSTIC CHARACTERISTICS
Triglycerides are a form of fatty esters. They are produced in the liver by binding glycerol and other fatty acids. Theyare transported by VLDL and LDL and act as a storage source for energy. Increased levels are found in hyperlipidemias, diabetes, nephrotic syndrome, hypothyroidism. Increased levels are risk factor for arteriosclerotic coronary disease and peripheral vascular disease. Decreased levels are found in malnutrition and hyperthyroidism. Triglycerides kit uses GPO / PAP method to determine triglycerides in serum or plasma

PRINCIPLE OF THE METHOD
Serum triglycerides are hydrolyzed to glycerol and free fatty acids by lipoprotein lipase. In the presence of ATP and glycerol kinase (GK), the glycerol is converted to glycerol-3-phosphate, which then is oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide. The oxidative condensation of 4-Chlorophenol and (4-AAP) 4-aminophenazone in the presence of Peroxidase (POD) and hydrogen peroxide produces a rose colored dye which is measured at 550 nm. The intensity of the colour formed is directly proportional to the triglycerides concentration in the sample.

COMPOSITION

REAGENT(R)	pH 7,2
Good's Buffer	50 mmol/l
4-Chlorophenol	4 mmol/l
ATP	2 mmol/l
Mg2+	15 mmol/l
Glycerokinase (GK)	≥ 0,4 kU/l
Peroxidase (POD)	≥ 2 kU/l
Lipoproteinlipase (LPL)	≥ 4 kU/l
4-Aminoantipyrine	0,5 mmol/l
Glycerin-3-phosphatoxidase	≥ 1,5 kU/l
STANDARD (S)	200 mg/dl

STORAGE
Store at 2-8ºC.
Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

REAGENT PREPARATION
Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT
− Thermostatic water bath at 37ºC
− Analyzer, spectrophotometer able to read at 546 nm

SAMPLES
Serum or plasma collected by standard procedures.
Cholesterol is stable for 7 days at 2-8ºC.
Heparin, EDTA, oxalate and fluoride may be used as anticoagulants

PROCEDURE
1. Bring the Reagent to room temperature.
2. Pipette into labeled test tubes:

	Blank	Standard	Sample
Reagent (R)	1.0 mL	1.0 mL	1.0 mL
Standard (S)	---	10 µL	---
Sample	---	---	10 µL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25ºC) or for 5 minutes at 37ºC.
4. Measure the absorbance (A) of the Standard and Sample at 546 nm against the Blank. The color is stable for at least 1 hour.

CALCULATIONS
The Triglycerides concentration in the sample is calculated using the following general formula:

$$\frac{\text{A Sample}}{\text{A Standard}} \times 200 = \text{mg/dL Triglycerides}$$
$$\frac{\text{A Sample}}{\text{A Standard}} \times 0.0114 = \text{mmol/L Triglycerides}$$

REFERENCE VALUES

Normal	Up to 150 mg/dL = 1.7 mmol/L
Borderline High	150-199 mg/dL = 1.70-2.25 mmol/L
High	200-499 mg/dL = 2.26-5.64 mmol/L
Very High	> 500 mg/dL = > 5.65 mmol/L

QUALITY CONTROL
It is recommended to use the Genesis Control Serum level Iand II to verify the performance of the measurement procedure.
Each laboratory should establish its own internal Quality Control

METROLOGICAL CHARACTERISTICS
- Detection limit: 4.4 mg/dL = 0.05 mmol/L.
- Linearity limit: 600 mg/dL = 6.78 mmol/L.
. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run):

Mean Concentration	**CV**	**n**
44 mg/dL = 0.50 mmol/L	2.8 %	20
207 mg/dL = 2.34 mmol/L	1.6 %	20

− Reproducibility (run to run):

Mean Concentration	**CV**	**n**
44 mg/dL = 0.50 mmol/L	2.9 %	25
207 mg/dL = 2.34 mmol/L	2.7 %	25

– INTERFERENCES:
Hemoglobin (10 g/L) does not interfere. Bilirubin (2.5 mg/dL) may interfere. Other drugs and substances may interfere

BIBLIOGRAPHY
1. Bucolo G and David H. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem 1973; 19: 476-482.
2. Fossati P and Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28: 2077-2080.
3. National Cholesterol Educarion Program Expert Panel. Third report of the Nationa Cholesterol Education Program (NCEP) Expert Panel onDetection, Evaluation, andTreatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda: NationalHeart, Lung, and Blood Institute; 2001.

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