H-1117 Budapest,
Budafoki street 111-113
Tel.: +36-1-205-3617
Tel./Fax: +36-1-205-3616

TRIGLYCERIDES PAP

STABLE LIQUID REAGENT

CE

Cat. No.:	47161,47161S	47162,47162S	47163
	120 ml	600 ml	20x20 ml

Reagent kit for the quantitative determination of triglycerides concentration in serum based upon enzymatic colorimetric method (PAP).

Triglycerides are esters formed from Glycerol and Fatty acids, the latter being synthesized in the liver or extracted from blood. Determining the level of Triglyceride concentration is part of the evaluation of lipid metabolism and plays a major role in identification of the various hyperlipoproteinemia. The level is increased in certain liver and renal diseases in diabetes mellitus and coronary artery disease.

Principle

The Triglycerides in the sample are hydrolyzed to Glycerol and Fatty acids by Lipoprotein lipase (LPL). Glycerine is then phosphorylated by Glycerol kinase (GK) in the presence of ATP and Mg^{++} ions. In the next step Glycerol-3-P is oxidized by Glycerol-3-Phosphate oxidase (GPO) in the presence of molecular oxygen (O_2). A colored product which absorbance well at 505 nm (490-550 nm) is formed from hydrogen-peroxide, 4-aminoantipyrine and phenol-derivative in the presence of the Peroxidase (POD).

$$Triglycerides + H_2O \xrightarrow{LPL} Glycerol + Fatty\ acids$$

$$Glycerol + ATP \xrightarrow{GK+Mg^{++}} G1ycerol\text{-}3\text{-}phosphate + ADP$$

$$Glycerol\text{-}3\text{-}phosphate + O_2 \xrightarrow{GPO} Dihydroxiacetone\text{-}phosphate + H_2O_2$$

$$2H_2O_2 + 4Aminoantipyrine + P\text{-}Chlorophenol \xrightarrow{POD} Red\ quinone + 4H_2O$$

Reference values

Female: **0.55-1.60 mmol/l** (40-140 mg/dl)
Male: **0.65-1.85 mmol/l** (50-165 mg/dl)
It is recommended that each laboratory should assign its own normal range.

Reagents

1. Reagent (R1)

Pipes buffer, pH=6,30	50 mmol/l
4-Chlorophenol	4 mmol/l
Mg2+	15 mmol/l
ATP	2 mmol/l
Glycerol kinase (GK)	0.4 KU/l
Peroxidase	2 KU/l
Lipoprotein lipase	2 KU/l
4-Aminoantipyrine	0.4 mmol/l
Glycerol-3-phosphate-Oxidase (GPO)	1.5KU/l

2. Triglycerides standard
Ready for use.For details please check the insert.
Available only in Cat. No.: 47161S and 47162S

Sample
Serum free of haemolysis.

Precautions
Discard cloudy reagent. Avoid contamination by using clean laboratory materials (pipettes, plastic vials for analyzers…).
The reagents contain 0.1 % sodium azide. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

PROCEDURE

Preparation and stability of working reagent
The reagents are ready for use. Avoid contamination of the opened reagents.
If the absorbance of working reagent is higher than 0.35 at 492 nm the reagent can not be used.

Assay conditions

Wavelength:	505 (490-550) nm (546Hg)
Temperature:	37 °C
Cuvette:	1 cm light path
Read against:	reagent blank
Method:	endpoint (increasing)

Pipette into cuvette

	Blank	Standard	Sample
Working reagent	1 ml	1 ml	1 ml
Distilled water	10µl		
Standard		10µl	
Sample			10µl

Mix and read the absorbance (A) after 5 minute at 37°C.

Calibration (37°C, GPO-PAP method)
S1: Distilled water
S2: Triglycerides standard Cat. No.: 51011 or
Roche C.F.A.S. (Calibrator for automated system)
Randox Calibration Serum Level I or
Randox Calibration Serum Level II

Calibration frequency
Two point calibration is recommended
- after reagent lot change,
- as required following quality control procedures.

Calculation

$$\frac{A_{sample}}{A_{standard}} xC_{standard} = C_{sample}$$

A = Absorbance, C = Concentration

Quality control
A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

PERFORMANCES DATA

The following data were obtained using the Olympus 400 analyzer (37°C).

Linearity
The test is linear from 0,006 (0,53 mg/dl) up to 11,27 mmol/l (1000 mg/dl).

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.0015 Abs is equivalent to 0.011 mmol/l (0,97mg/dl) triglycerides concentration at 505 nm.

Precision

	Reproducibility		
	Average concentration (mmol/l)	SD	CV%
Sample I	1,44	2,01	1,57
Sample II.	2,22	1,961	0,996

	Repeatability		
	Average concentration (mmol/l)	SD	CV%
Sample I	1,43	4,128	3,155
Sample II.	2,28	15,56	7,704

Correlation
Comparative studies were done to compare our reagent with another commercial Triglycerides PAP reagent.
The results from these studies are detailed below.
Correlation coefficient: r = 0,9917
Linear regression: y (mmol/l) = 0,8843x+0,1547
(x= other commercial reagent, y= own reagent).

Specificity

Bilirubin up to 170 µmol/l (10 mg/dl), hemoglobin up to 10 g/l don't interfere with the assay up to the given levels.

NOTE
Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For *in vitro* diagnostic use only.

The following symbols are used on labels

IVD For in vitro diagnostic use

Use by (last day of the month)

Temperature limitation

LOT Batch Code

REF Code

Bibliography
Buccolo G., David M., ClinChem., 19,476 1973;
Werner M., Gabrielson D.G., Eastman G., Clin. Chem, 21, 268,1981;
Annoni G., Bottasso B.M., Ciaci D., Donato M.F., Tripoli A., 1ab JJ. Res. Lab. Med. 9 115, 1982;