**Triglycerides. GPO-POD. Enzymatic colorimetric** Ref.:TRI-014
*Quantitative Determination of triglycerides*
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC 2 x 125 mL

# TRIGLYCERIDES

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## PRINCIPLE OF THE METHOD

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide ($H_2O_2$).
In the last reaction, hydrogen peroxide ($H_2O_2$) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{p-Chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the triglycerides concentration in the sample[1,2,3].

## CLINICAL SIGNIFICANCE

Triglycerides are fats that provide energy for the cell.
Like cholesterol, they are delivered to the body's cells by lipoproteins in the blood. A diet with a lot of saturated fats or carbohydrates will raise the triglyceride levels. The increases in serum triglycerides are relatively non-specific. For example liver dysfunction resulting from hepatitis, extra hepatic biliary obstruction or cirrhosis, diabetes mellitus is associated with the increase [3,6,7].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

## REAGENTS

| | | |
|---|---|---|
| **R 1** Buffer | GOOD pH 7.5 | 50 mmol/L |
| | p-Chlorophenol | 2 mmol/L |
| **R 2** Enzymes | Lipoprotein lipase (LPL) | 150000 U/L |
| | Glycerolkinase (GK) | 500 U/L |
| | Glycerol-3-oxidasa (GPO) | 2500 U/L |
| | Peroxidase (POD) | 440 U/L |
| | 4 – Aminophenazone (4-AP) | 0.1 mmol/L |
| | ATP | 0.1 mmol/L |
| **TRIGLYCERIDES CAL** | Triglycerides aqueous primary standard 200 mg/dL | |

## PREPARATION

Working reagent (WR): Dissolve ( → ) the contents of one vial R 2 Enzymes into one bottle of R 1 Buffer.

Ref: 1001310 Working reagent (WR): Dissolve ( → ) the contents of one vial R 2 Enzymes in 10 mL of R 1 Buffer.

Cap and mix gently to dissolve contents.
WR stability: 6 weeks at 2-8ºC or 1 week at room temperature (15-25ºC).

## STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

## TRIGLYCERIDES CAL

Once open is stable up to 1 month when stored tightly closed at 2-8ºC, protected from light and contaminations prevented during their use.

**Signs of reagent deterioration:**
- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm ≥ 0.14.

## ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 505 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

## SAMPLES

Serum or heparinized or EDTA plasma[1]. Stability of the sample: 5 days at 2-8ºC .

## PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . 505 nm (490-550)
   Cuvette: . . . . . 1 cm light path
   Temperature . . . . . 37ºC / 15-25ºC
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

| | Blank | Standard | Sample |
|---|---|---|---|
| WR (mL) | 1.0 | 1.0 | 1.0 |
| Standard(Note 1,2) (µL) | -- | 10 | -- |
| Sample (µL) | -- | -- | 10 |

4. Mix and incubate for 5 min. at 37ºC or 10 min. at room temperature.
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

## CALCULATIONS

$\frac{(A)\text{Sample}}{(A)\text{Standard}}$ x 200 (Standard conc.) = mg/dL triglycerides in the sample

**Conversion factor:** mg/dL x 0.0113= mmol/L.

## QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

## REFERENCE VALUES

| | |
|---|---|
| Men | 40 – 160 mg/dL |
| Women | 35 – 135 mg/dL |

These values are for orientation purpose; each laboratory should establish its own reference range.

## PERFORMANCE CHARACTERISTICS

*Measuring range:* From *detection limit* of 0.7 mg/dL to *linearity limit* of 1000 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
*Precision:*

| | Intra-assay (n=20) | | Inter-assay (n=20) | |
|---|---|---|---|---|
| Mean (mg/dL) | 118 | 216 | 119 | 215 |
| SD | 0.67 | 0.94 | 2.17 | 2.91 |
| CV (%) | 0.60 | 0.43 | 1.83 | 1.36 |

Sensitivity: 1 mg/dL = 0.0012 A.
*Accuracy*: Results obtained using BSM reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.996.
Regression equation: y= 1.00x + 0.0743.
The results of the performance characteristics depend on the analyzer used.

## INTERFERENCES

No interferences were observed with bilirubin up to 170 µmol/L and hemoglobin up to 10 g/L[2].
A list of drugs and other interfering substances with cholesterol determination has been reported by Young et al [4, 5].

## NOTES

1. LCF (Lipid Clearing Factor) is integrated in the reagent.
2. Calibration with the aqueous Standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.
4. **BSM has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

## BIBLIOGRAPHY


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