Reactivos GPL

Barcelona, España

CE

-TRIGLYCERIDES-

TRIGLYCERIDES
GPO-POD. Enzymatic-Colorimetric

Store at: +2+8ºC.

Presentation:
Cod. SU035-LYO CONT: *R 9 x 50 mL.+ CAL 1 x 5 mL.*

Procedure

Quantitative determination of triglycerides.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

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 (GK) and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate oxidase (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:

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

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

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

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

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

REAGENTS COMPOSITION

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

REAGENT PREPARATION AND STABILITY

Working Reagent (WR): Dissolve (→) the contents of one vial R.2 (Enzymes) in one bottle R.1 (Buffer). Cap and mix gently to dissolve contents. (WR) is stable: 6 weeks at 2-8ºC or 1 week at 15-25ºC.
TRIGLYCERIDES CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 505 nm. ≥ 0.14

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.

SPECIMEN

Serum or plasma[1]. Stability of the sample: 5 days at 2-8º C.

MATERIAL REQUIRED BUT NOT PROVIDED

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

General laboratory equipment.

TEST PROCEDURE

1. Assay Conditions
   - Wavelength: ..... 505 nm. (490-550).
   - Cuvette: ..... 1 cm light path.
   - Temperature .....37º C. 15-25º C.
2. Adjust the instrument to zero with Blank of reagent.
3. Pipette into a cuvette (Note 3):

	Blank	Standard	Sample
R (mL.)	1.0	1.0	1.0
Calibrator (note 2) (µL.)	--	10	--
Sample (µL.)	--	--	10

4. Mix and incubate for 5 minutes at 37ºC or 10 minutes at room temperature (15-25ºC).
5. Read the absorbance (A) of the samples and calibrator, against the Blank. The colour is stable at least 30 minutes.

CALCULATIONS

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

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, H Normal (QC003) and H Pathological (QC004).
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES

Men: 40 – 160 mg/dL.
Women: 35 – 135 mg/dL.

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

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 triglycerides 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.

REAGENT PERFORMANCE

– *Measuring Range*:
From detection limit of 0.000 mg/dL. to linearity limit of 1200 mg/dL., under the described assay conditions.
If results obtained were greater than linearity limit, dilute the sample ½ with NaCl 9 g/L. and multiply result by 2.

– *Precision*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (mg/dL)	103	219	103	217
SD	0.41	0.93	3.74	7.80
CV %	0.39	0.43	3.62	3.59

- *Sensitivity:* 1 mg/dL. = 0.0037 A
- *Accuracy:* Results obtained GPL 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 $(r)^2$: 0.99760
  Regression Equation: y= 0.905x + 10.77
  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES

- No interferences were observed to bilirubin up to 170 µmol/L, hemoglobin up to 10 g/L[1,2].
- Other substances may interfere. A list of drugs and other substances that could interfere has been reported[3,4].

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.

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