| COD 11828<br>1 x 50 mL | COD 11528<br>4 x 50 mL | COD 11529<br>2 x 250 mL |
|---|---|---|
| STORE AT 2-8ºC | | |
| Reagents for measurement of triglycerides concentration<br>Only for *in vitro* use in the clinical laboratory | | |

**TRIGLYCERIDES**

BioSystems

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**TRIGLYCERIDES**

GLYCEROL PHOSPHATE OXIDASE/PEROXIDASE

## PRINCIPLE OF THE METHOD

Triglycerides in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1,2].

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{glycerol kinase}} \text{Glycerol} - 3 - P + ADP$$

$$\text{Glycerol} - 3 - P + O_2 \xrightarrow{\text{G-3-P-oxidase}} \text{Dihidroxyacetone} - P + H_2O_2$$

$$2\ H_2O_2 + 4 - \text{Aminoantipyrine} + 4 - \text{Chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

## CONTENTS

| | COD 11828 | COD 11528 | COD 11529 |
|---|---|---|---|
| A. Reagent | 1 x 50 mL | 4 x 50 mL | 2 x 250 mL |
| S. Standard | 1 x 5 mL | 1 x 5 mL | 1 x 5 mL |

## COMPOSITION

A. Reagent: Pipes 45 mmol/L, magnesium acetate 5 mmol/L, 4-chlorophenol 6 mmol/L, lipase > 100 U/mL, glycerol kinase > 1.5 U/mL, glycerol-3-phosphate oxidase > 4 U/mL, peroxidase > 0.8 U/mL,4-aminoantipyrine 0.75 mmol/L, ATP 0.9 mmol/L, pH 7.0.

S. Triglycerides Standard: Glycerol equivalent to 200 mg/dL (2.26 mmol/L) triolein. Aqueous primary standard.

## 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.

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

## REAGENT PREPARATION

Reagent and Standard are provided ready to use.

## ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC.
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

## SAMPLES

Serum or plasma collected by standard procedures.

Triglycerides in serum or plasma are stable for 5 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 labelled test tubes: (Note 1)

| | Blank | Standard | Sample |
|---|---|---|---|
| Triglycerides Standard (S) | — | 10 µL | — |
| Sample | — | — | 10 µL |
| Reagent (A) | 1.0 mL | 1.0 mL | 1.0 mL |

3. Mix thoroughly and incubate the tubes for 15 minutes at room temperature (16-25ºC) or for 5 minutes at 37ºC.
4. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 2 hours.

## CALCULATIONS

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

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

If the Triglycerides Standard provided has been used to calibrate (Note 2):

| | |
|---|---|
| $\frac{A_{Sample}}{A_{Standard}}$ | x 200 = mg/dL triglycerides<br>x 2.26 = mmol/L triglycerides |

## REFERENCE VALUES

The following uniform cut-off points have been established by the US National Institutes of Health and have also been adopted in many other countries for the evaluation of risk[3].

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

## QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

## METROLOGICAL CHARACTERISTICS

- Detection limit: 1.6 mg/dL = 0.018 mmol/L
- Linearity limit: 600 mg/dL = 6.78 mmol/L. For higher values dilute sample 1/ 4 with distilled water and repeat measurement.
- Repeatibility (within run):

| Mean Concentration | CV | n |
|---|---|---|
| 100 mg/dL = 1.13 mmol/L | 1.7 % | 20 |
| 245 mg/dL = 2.77 mmol/L | 0.7 % | 20 |

- Reproducibility (run to run):

| Mean Concentration | CV | n |
|---|---|---|
| 100 mg/dL = 1.13 mmol/L | 2.6 % | 25 |
| 245 mg/dL = 2.77 mmol/L | 1.7 % | 25 |

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemolysis (hemoglobin up to 1000 mg/dL), bilirubin (up to 2.5 mg/dL) do not interfere. Ascorbic acid (up to 5 mg/dL) does not interfere.Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

## DIAGNOSTIC CHARACTERISTICS

Triglycerides are esters of glycerol and fatty acids coming from the diet or obtained by synthesis mainly in the liver. Triglycerides are transported in plasma by lipoproteins and used by adipose tissue, muscle and other. Their primary function is to provide energy to the cell.

Elevated serum triglycerides levels can be caused by liver disease, diabetes mellitus, nephrosis, hypothyroididsm, alcoholism, familial hyperlipoproteinemia IV and V, and other[3,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

## NOTES

1. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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