



GLYCEROL

COLORIMETRIC DETERMINATION IN BIOLOGICAL FLUIDS

Kit: 4 x 100 ml

Code TG8840

SUMMARY

Glycerol is the most common alcohol present in humans. It is the most important part of the Acylglycerols (Glycerol Esters or Glycerides), Triglycerides are the most prevalent glycerol esters we meet in the human nutrition.

Triglycerides represent 95% of tissue storage fat and are the predominant form of glycerol esters found in plasma. Triglycerides are digested in the duodenum and proximal ileum; by the action of pancreatic and intestinal lipases, in the presence of bile acids, they are hydrolyzed to glycerol, monoglycerides and fatty acids. After demolition and absorption, triglycerides are resynthesized in the intestinal epithelial cells.

PRINCIPLE

Glycerol is phosphorylated to glycerol-3-phosphate by glycerokinase, then converted into dihydroxyacetonephosphate and hydrogen peroxide by glycerol-3-phosphate oxidase.

Hydrogen peroxide reacts in presence of peroxidase with 4-aminophenazon and TOOS forming a red-purple quinone compound, intensity of colour is proportional to the concentration of GLYCEROL in the sample.

REAGENTS

Components of the kit:

***REAGENT 1 (liquid)**

***REAGENT 2 (Iyo)**

GK ≥ 100 U/L
GPO ≥ 4000 U/L
POD ≥ 1000 U/L
ATP 2 mmol/L
4-aminophenazon >0.1 mmol/L
GOOD Buffer 0.1 M
TOOS > 0.3 mmol/L

***REAGENT 3 (liquid) Standard**

Glycerol 20,8 mg/dL = 0.208 g/L = 208 mg/L

Code TG8840

4 x 100 ml

4 x 100 ml

1 x 5 ml

STABILITY: the reagents, stored at 2-8°C, are stable up to the expiry date shown on the package **if not contaminated during handling.**

PREPARATION OF THE WORKING REAGENT

Dissolve a vial of *Reagent 2 with 100 ml of *Reagent 1 and mix gently till dissolution. Please avoid foaming.

A suggestion could be to aliquote in vials the quantity for each stage of analysis; to put the need for the day at 2-8°C for use, to freeze the remaining vials for next stages.

Let the reagents reach the working temperature before use.

Close immediately after handling.

Incompetent handling will release us from any responsibility.

STABILITY: the diluted *Reagent 2 is stable 30 days at 2-8°C.

Till 6 months frozen at -20°C. FREEZE only ONE TIME.

DO NOT REPEAT FREEZING.

SAMPLE

- Please use limpid sample, at a NEUTRAL pH.
- If necessary, filter the sample.
- We strong suggest to neutralize the Acidic Samples at a neutral pH (max pH ≤ 8,0).
- If tested in Plasma, use EDTA.

PROCEDURE

- Wavelength: 550 nm (530-570 nm)
- Pathlength: 1 cm
- Reading: against air or distilled water
- Temperature: 37°C
- Method: end-point
- Reaction: 5 - 10 minutes
- Linearity: 2 - 500 mg/L at 37°C as Glycerol
- Sample/reagents: 1/100

Let reagents reach the working temperature before using.

Pipette in 3 test tubes so labelled:

R/B: Reagent Blank, S: Sample, ST: Standard:

	R/B	S	ST
Working reagent	1000 µl	1000 µl	1000 µl

Allow the reagent to reach 37°C and add:

*Reagent 3 Standard	----	----	10 µl
Sample	----	10 µl	----
Distilled water	10 µl	----	----

Mix carefully and incubate at 37°C for 5-10 minutes, waiting the end of the reaction. Read the absorbance of the standard (Ast) and of the sample (As) against the Reagent Blank.

Final colour is stable for 60 minutes at room temperature in the dark.

CALCULATION

$$(As/Ast) \times 20.8 = \text{mg / dL}$$

$$(As/Ast) \times 0.208 = \text{g / L}$$

$$(As/Ast) \times 208 = \text{mg / L}$$

REFERENCE VALUES

Glycerol

3 – 10 years 5,6 – 21,4 mg/L

11 – 80 years 2,9 – 17,2 mg/L

It is suitable that every laboratory determine its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: The Glycerol Reagent is linear to 500 mg/L.

For concentrations ≥ 500 mg/L, dilute the sample 1:4 with saline sol., repeat the determination and multiply the result x 4.

Sensitivity: The minimum detectable is 2 mg/L.

Within-run Precision:

	Mean (mg/L) ± 2s	CV %
Serum 1	15 ± 0,5	1,9
Serum 2	400 ± 4	0,6

Run-to-run (Day-to-day) Precision:

	Mean (mg/L) ± 2s	CV %
Serum 1	15 ± 0,7	2,1
Serum 2	400 ± 6	0,8

Interferences: See References point 2.

Correlation: A group of 20 sera from 15 to 500 mg/L was assayed by this procedure and using a similar commercially available Glycerol Reagent.

Comparison of the data gave following results:

$$\text{Linear regression equation } y = 1,007x - 0,3$$

$$\text{Correlation coefficient } r = 0,9987$$

NOTE

1. A proportional variation of the reaction volumes does not change the result.
2. We suggest do not mix Reagents from different Production lots.
3. For concentrations higher than 500 mg/L dilute the sample 1:4 with saline solution, repeat the determination and multiply the result by 4.
4. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Glycerol (see References 2.).
5. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
6. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
7. The clinical diagnosis cannot be do using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975).

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