

GLYCEROL

ENZYMATIC COLORIMETRIC DETERMINATION IN WINE, FOOD & BEVERAGES

Kit: 4 x 100 mL

Code TG9940

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-aminophenazone 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-aminophenazone	>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

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 aliquot 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

- Wine could be used directly.
- Use colourless, clear and quite neutral liquid samples directly if Glycerol conc. is between 0.020–0,500 g/L; otherwise, dilute with water to reduce it in this range.
- Turbid solutions have to be filtered or centrifuged
- Samples containing carbon dioxide have to be degased.
- Acid samples have to be adjusted by adding KOH /NaOH until approx. pH 8 is reached.
- Alkaline samples have to be adjusted by adding HCl until approx. pH 8 is reached.
- Strongly coloured samples have to be treated with PVPP (polyvinylpyrrolidone e.g. 1 g/100 mL Sample).
- For other different samples, please inquire the use and for potential pre-treatment.

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: 20 - 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 labeled:

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

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

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

NOTE

1. A prop. variation of the reaction volumes does not change the results.
2. We suggest do not mix Reagents from different Production lots.
3. For concentrations higher than the limit of Linearity of the different applications, dilute the sample with distilled water in the mentioned ranges; repeat the determination and multiply the result by the dilution factor.
4. **PAY ATTENTION!** Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
5. For fat containing samples please ask for specific procedure.
6. For solid or semi-solid samples please ask for specific procedure, eventual Carrez solutions pretreatment and Calculation.
7. Specificity: this test is specific for Glycerol. No interference was seen.

Ver. 2007/10