

Enzymatic kit for determination of Glycerol

Principle:

$$ADP + PEP \xrightarrow{PK} ATP + pyruvate$$

The determination is based on the formation of NAD* measured by the decrease in light absorbance at 340 nm

Kit for 45 / 135 analysis includes

Bottle	Composition	Quantity
A.	Buffer	100 ml
В.	Coenzymes	10 ml
C.	Enzymes	1000 µl
D.	Diluent	13 ml
STD.	Standard 1 g/l	2 ml

Reagent preparation:

R1: preparation for 9 analysis with macro cuvettes (or 27 analysis with semi-micro cuvettes)

Mix gently Bottle B. Combine 2 ml of Bottle B with 18 ml of Bottle A.

Stability of R1: 1 week if stored at 2-8 °C.

R2: preparation for 9 analysis with macro cuvettes (or 27 analysis with semi-micro cuvettes)

Mix gently Bottle C . Combine 200 μ l of Bottle C with 2600 μ l of Bottle D.

Stability of R2: 4 weeks if stored at 2-8 °C.

Sample preparation:

If necessary, filtrate or centrifuge the sample in order to eliminate the particles in suspension.

Performances:

This test has been developed to determine the concentration of glycerol in a measuring range from 0 to 1 g/l.

If the expected values are higher than 1 g/l, samples should be diluted with distilled water and the results must be multiplied by the dilution factor.

Storage instructions and reagent stability:

The reagents are stable up to the expiry date, if stored at 2 to 8°C. Contamination should be avoided. Do not freeze the reagents!

Warnings and precautions:

Do not swallow the reagents. Avoid contact with the skin and mucous membranes.

Sample analysis

	Blank	Standard	Sample
R1	2200 μΙ	2200 µl	2200 μΙ
Water	30 μΙ		
Standard		30 μΙ	
Sample			30 μΙ
Mix and read	DO1 blank	DO1 standard	DO1 sample
R2	300 μΙ	300 μΙ	300 μΙ
Mix wait 15 min and read	DO2 blank	DO2 standard	DO2 sample

Wavelength: 340 nm Cuvette: 1 cm path (plastic or glass) Temperature: 20 - 37°C

Zero: against water or air

Calculations:

ΔDO sample = (DO2-DO1) sample - (DO2-DO1) blank

 ΔDO standard = (DO2-DO1) standard - (DO2-DO1) blank

C sample (g/l) = C standard x $\frac{\Delta DO \text{ sample}}{\Delta DO \text{ standard}}$

Dilution factor of the sample has to be considered in the calculation

It's possible to perform 135 analysis if working with semi-micro and applying the volumes below: R1= 730 µl / Sample = 10 µl / R2= 100 µl