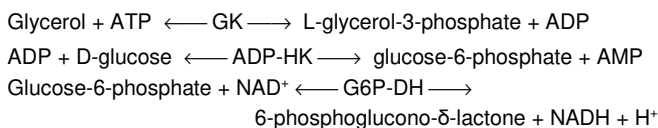


Enzymatic assay for the determination of glycerol in foodstuff and other sample materials  
2 x 50 ml R1 and 2 x 12.5 ml R2 – 50 assays (manual) / ≥ 500 assays (auto-analyzer)

For *in vitro* use only  
Store between 2 - 8 °C

**Principle**

Enzymatic UV test with glycerokinase (GK), ADP-dependent hexokinase (ADP-HK) and Glucose-6-phosphate dehydrogenase (G6P-DH). Glycerol is phosphorylated by ATP and glycerokinase to L-glycerol-3-phosphate plus ADP. D-glucose is then phosphorylated by ADP-HK to glucose-6-phosphate. In the presence of G6P-DH, glucose-6-phosphate is oxidized with production of NADH:



The amount of NADH formed is equivalent to the initial amount of glycerol and is measured at 340 nm.

**Reagents**

The reagents are ready-to-use.

- Reagent 1: 2 x 50 ml (Buffer / NAD)
- Reagent 2: 2 x 12.5 ml (GK, ADP-HK, G6P-DH)

The reagents are stable up to the end of the indicated month of expiry if stored at 2 - 8 °C, even after repeated opening (if not contaminated during handling). Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate material safety data sheets (MSDS) for this product, available online at [www.r-biopharm.com](http://www.r-biopharm.com). After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

**Sample preparation**

- Use liquid and clear samples directly or after dilution into the relevant measuring range (see test performance)
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- Clarify samples containing proteins with Carrez clarification
- Crush and homogenize solid or semi-solid samples and extract with water (e.g. 30 min at 60 - 70°C). Filter or centrifuge, or apply Carrez clarification if necessary.
- For fat containing samples, extract with hot water, cool down to separate the fat (fridge or ice), remove the fatty layer and filter the aqueous part

**Assay procedure**

Wavelength: 340 nm  
Optical path: 1 cm  
Temperature: 37 °C / 20 - 25 °C  
Measurement: Against air or against water

	Reagent blank	Samples / Controls
<b>Reagent 1</b>	2000 µl	2000 µl
<b>Sample / Control</b>	-	100 µl
<b>Dist. water</b>	100 µl	-
Mix, incubate for 1 min at 37 °C or 3 min at 20 - 25 °C. Read absorbance A <sub>1</sub> , then add:		
<b>Reagent 2</b>	500 µl	500 µl
Mix, incubate 5 min at 37°C or 10 min at 20 - 25 °C. Read absorbance A <sub>2</sub> .		

The reagent blank must be performed once for each run and subtracted from each sample result.

**Calculation of results**

**Calculation of sample solutions:**

$$\Delta A = (A_2 - df \times A_1)_{\text{sample}} - (A_2 - df \times A_1)_{\text{RB}}$$

df: dilution factor  
RB: Reagent blank

$$df = \frac{(\text{sample volume} + R1)}{(\text{sample volume} + R1 + R2)} = 0.808$$

$$c_{\text{Glycerol}} [\text{g/l}] = \frac{(V \times MW \times \Delta A)}{(\epsilon \times d \times v \times 1000)}$$

V: Total volume [ml] = 2.600  
MW: Molecular weight [g/mol] = 92.10  
d: Optical path [cm] = 1.00  
v: Sample volume [ml] = 0.100  
ε: Extinction coefficient NADH [l/mmol x cm] = 6.3 (at 340 nm)

For a determination at 340 nm this results in:

$$c_{\text{Glycerol}} [\text{g/l}] = 0.3801 \times \Delta A$$

**Calculation of solid samples:**

$$\text{Content}_{\text{Glycerol}} [\text{g}/100 \text{ g}] = \frac{c_{\text{Glycerol}} [\text{g/l}]}{\text{weight}_{\text{sample}} [\text{g/l}]} \times 100$$

**Performance data**

**Specificity**

The test is specific for glycerol. Dihydroxyacetone (< 0.3 g/l) and D-glucose/D-fructose or sucrose (< 50 g/L) have no significant influence on the measurement result.

**Linearity & Measuring range**

Linearity is given up to 1 g/l glycerol. The recommended measuring range is between 8 and 800 mg/l glycerol. If this range is exceeded, the samples should be diluted with dist. water to a glycerol concentration within the measuring range. The dilution factor must be taken into account in the calculation.

**Sensitivity**

The Limit of Detection (LoD) and Limit of Quantification (LoQ) were determined according to the method DIN 32645:2008-11 in buffered aqueous solution:

- LoD = 4.0 mg/l
- LoQ = 8.0 mg/l

**Automation**

Application sheets for automated systems are available on request.

**Disclaimer**

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