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:

Glycerol + ATP ⟵ GK ⟶ L-glycerol-3-phosphate + ADP

ADP + D-glucose ⟵ ADP-HK ⟶ glucose-6-phosphate + AMP

Glucose-6-phosphate + NAD^+ ⟵ G6P-DH ⟶
6-phosphoglucono-δ-lactone + NADH + H^+

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. 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**
Reagent 1	2000 µl	2000 µl
Sample / Control	-	100 µl
Dist. water	100 µl	-
Mix, incubate for 1 min at 37 °C or 3 min at 20 - 25 °C. Read absorbance A_1, then add:		
Reagent 2	500 µl	500 µl
Mix, incubate 5 min at 37°C or 10 min at 20 - 25 °C. Read absorbance A_2.		

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

Calculation of results

Calculation of sample solutions:

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

df: dilution factor
RB: Reagent blank

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

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

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:

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

Calculation of solid samples:

$$Content_{Glycerol}\,[g/100\,g] = \frac{c_{Glycerol}\,[g/l]}{weight_{sample}\,[g/l]} \times 100$$

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) where 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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