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

Method & Principle

Enzymatic UV test with glycerokinase (GK), ADP-hexokinase (ADP-HK) and Glucose-6-phosphate dehydrogenase (G6P-DH).

Catalyzed by glycerokinase, glycerol is phosphorylated by ATP to L-glycerol-3-phosphate. The resulting ADP and D-glucose are then phosphorylated by an ADP-dependent hexokinase to glucose-6-phosphate. In the presence of glucose-6-phosphate dehydrogenase, glucose-6-phosphate is oxidized to 6-phosphoglucono-δ-lactone and 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 NADH consumption is stoichiometric with the amount of glycerol, which is measured by an increase of absorbance 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. 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, clear and nearly neutral samples directly or after dilution into the relevant measuring range (see test performance)
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- In general, aqueous extraction is recommended for the determination of glycerol (e.g. 30 min at 60 °C).
  Crush and homogenize solid and semi-solid samples, weigh suitable sample amount into a volumetric flask and extract with water. If necessary, filter or apply Carrez clarification.
- Weigh heavily fatty samples in suitable quantities into a volumetric flask, extract with hot water; allow cooling for fat separation (e.g. refrigerator or ice bath); remove fat layer and finally filter.

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:

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

df: dilution factor
RB: Reagent blank

$$df = \frac{(\text{sample volume} + R1)}{(\text{sample volume} + R1 + R2)} \times 100 = 0.808$$

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

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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