UV assay for the determination of glycerol in foodstuffs and other sample materials
Test combination for 50 determinations

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

1. Test 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 GK to L-glycerol-3-phosphate plus ADP.

Glycerol + ATP $\xleftarrow{}$ GK $\xrightarrow{}$ L-glycerol-3-phosphate + ADP

The resulting ADP is converted with D-glucose by an ADP-dependent hexokinase (ADP-HK) to glucose-6-phosphate (G6P).

ADP + D-glucose $\xleftarrow{}$ ADP-HK $\xrightarrow{}$ glucose-6-phosphate + AMP

In the presence of G6P-DH, glucose-6-phosphate is oxidized with production of NADH to 6-phosphoglucono-δ-lactone.

Glucose-6-phosphate + NAD^+ $\xleftarrow{}$ G6P-DH $\xrightarrow{}$ 6-phosphoglucono-δ-lactone + NADH + H^+

Nicotinamide-adenine-dinucleotide (NAD) is reduced to NADH. The amount of NADH formed is proportional to the glycerol conversion and is measured at 340 nm.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

- Reagent 1: 2 x 50 mL with buffer, NAD
- Reagent 2: 2 x 12.5 mL with buffer, GK, ADP-HK, G6P-DH

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

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 safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- 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 °C). Filter or centrifuge, or apply Carrez clarification if necessary.
- Weigh samples with a high fat content into a volumetric flask and extract with hot water; allow sample solution to cool down for fat separation (e.g. 15 min in an ice bath); fill volumetric flask up to the mark with water, filter aqueous solution before testing.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 8 - 800 mg/L

	Reagent blank	**Samples / controls**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance A_1, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 10 min at 20 - 37 °C and read absorbance A_2.		

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

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of glycerol

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume + R1}}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume of very acidic samples is increased, the test system may be affected. This must be checked.

$$\mathbf{C_{glycerol}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.380 \times \Delta A}$$

V:	Test volume basic application [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)

5.2. Calculation of solid samples

$$Content_{glycerol}\ [g/100\ g] = \frac{C_{glycerol}\ [g/L\ sample\ solution]}{weight_{sample}\ in\ g/L\ sample\ solution} \times 100$$

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend the use of the Enzytec™ *Liquid* Multi-Sugar Standard low (E8440).
The recovery of Enzytec™ *Liquid* Multi-Sugar Standard low and other aqueous control solutions should be within 100 ± 5 % and for extracted food samples within 100 ± 10 %.

6. Performance data

6.1. Specificity & side activities

The test is specific for glycerol. A slight side activity of 5 % to dihydroxyacetone is given.

6.2. Interferences

All substances tested, except sulfite, ethanol and urea showed no interference. In case of sulfite, ethanol and urea, a dilution of the solutions to 1 g/L in distilled water is recommended.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 900 mg/L glycerol, with the recommended measuring range between 8 and 800 mg/L (sample volume of 100 µL).
The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11, using buffered aqueous solution. This results in an LoD of 2.0 mg/L. The limit of quantification (LoQ) was determined by precision profile and is 8.0 mg/L.
The smallest absorbance difference that the method can distinguish is ΔA = 0.005. For a sample volume of v = 1000 µL, this results in an LoD of 0.26 mg/L. Based on ΔA = 0.010, an LoQ of 0.51 mg/L was calculated.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

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