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

For *in vitro* use only
Store between 2 – 8 °C (36 – 46 °F)

This test was validated using selected samples of the following matrices: wine, beer, juices, honey, lotion, soap, and toothpaste.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

1. Test principle

Glycerol is phosphorylated by the enzyme glycerol kinase (GK) with adenosine 5'-triphosphate (ATP) to form L-glycerol-3-phosphate:

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

The resulting adenosine 5'-diphosphate (ADP) is converted with D-glucose by an ADP-dependent hexokinase (ADP-HK), to glucose-6-phosphate (G-6-P) and adenosine 5'-monophosphate (AMP):

ADP + D-glucose ⟵ ADP-HK ⟶ G-6-P + AMP

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), glucose-6-phosphate is converted to 6-phosphoglucono-δ-lactone:

G-6-P + NAD^+ ⟵G6P-DH⟶ 6-phosphoglucono-δ-lactone + NADH + H^+

Nicotinamide adenine dinucleotide (NAD) is reduced to NADH in this process. The amount of NADH formed is proportional to the amount of glycerol converted 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 / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

3.1. General

- Sample preparation for manual and automated testing is the same.
- Bring sample solutions to room temperature before measurement.
- Use liquid, clear, colorless and almost neutral sample solutions directly or dilute sufficiently to yield a glycerol concentration within the stated measuring range (refer to performance data).
- For turbid test samples: Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbonic acid, e.g. by stirring in a beaker, applying a brief ultrasonic burst, filtration or centrifugation.
- Crush and homogenize solid and semi-solid samples. Weigh a sufficient quantity of sample in a volumetric flask (considering the measuring range), extract with water; fill up to the mark and filter if necessary (by using fluted paper or syringe filters) or centrifuge in reaction tubes. Use Carrez clarification if necessary.
- For fat containing samples, weigh sufficient quantity (considering the measuring range) into a volumetric flask and extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 minutes and filter.
- Clarify samples containing proteins or fat with Carrez reagents: Weigh an appropriate sample quantity accurately into a 100 mL volumetric flask and add approx. 60 mL distilled water. In case of liquid samples, pipette the sample into a 100 mL volumetric flask or beaker pre-filled with 60 mL distilled water. Add 5 mL Carrez I solution (3.60 g potassium hexacyano-ferrate(II)-trihydrate $K_4[Fe(CN)_6]$ x 3 H_2O/100 mL) and 5 mL Carrez II solution (7.20 g zinc sulfate $ZnSO_4$ x 7 H_2O/100 mL). Mix well after each addition. Adjust the pH with 0.1 M NaOH to a value between 7.5 and 8.5. Transfer into a 100 mL volumetric flask, fill up to the mark, mix and filter using fluted paper filters or syringe filters.
- In case of higher sample volumes (up to 1000 µL), check the pH value of the test solution and neutralize in case of any doubt.

4. Manual test procedure

Wavelength:	340 nm
Temperature (measurement):	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
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 minutes at 20 – 37 °C (68 – 99 °F)**. **Read** absorbance **A_1**, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for **10 minutes at 20 – 37 °C (68 – 99 °F)** and **read** absorbance **A_2**.		

4.1. Important notes for assay procedure

- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Specified incubation times were validated at 25 °C (77 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.

- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- Always wait for the reaction to end or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped after the recommended incubation time, continue measuring at 5-minute intervals, for example, until a constant absorbance value is reached.
- If a creep reaction occurs, the reaction will not have finished after stated incubation times and will typically show a constant increase of ΔA. Calculate the analyte-specific ΔA value by plotting the absorbance values against time and performing a linear regression to determine the rate of increase in ΔA per minute related to the creep reaction. Then, extrapolate the absorbance to the time at which reagent 2 is added.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of glycerol

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

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

The specified df value of **0.808 applies to a base application of 100 µL**. An increase in sample volume is possible (max. 1000 µL; refer to validation report). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df) accordingly**.

Increasing the sample volume may influence test performance. This must generally be checked depending on the matrix. **The reagent blank value must be adjusted to the changed sample volume.**

The concentration of glycerolin sample solutions is calculated using Lambert-Beer's law:

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

If the sample solution was diluted before measurement, this result has to be multiplied with the **sample pre-dilution factor F**.

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight glycerol [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

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weight:

$$\text{Content}_{\text{glycerol}}\ [\text{g}/100\ \text{g}] = \frac{C_{\text{glycerol}}\ [\text{g/L sample solution}]}{\text{weight}_{\text{sample}}\ \text{in g/L sample solution}} \times 100$$

5.3. Controls & acceptance criteria

Control or reference samples should be included in each run for quality control purposes. Therefore, we recommend Enzytec™ *Liquid* Multi-Sugar Standard *low* (Art. No. E8440; 0.200 g/L glycerol).

The recovery of this standard and other *aqueous* control solutions should be 100 ± 5 %, for extracted food samples, it should be within the range of 90 – 110 %.

6. Performance data

6.1. Specificity & side activities

The test is specific for glycerol. A slight side activity of 5 % to dihydroxyacetone is given. Apart from that, the test shows no side-activity with other relevant acids.

6.2. Interferences

An interference study was conducted with various substances in the presence of 200 mg/L glycerol, and the respective recovery rates were determined. The substances tested included: ascorbic acid, SO_2, L-lactic acid, acetic acid, D-/L-tartaric acid, citric acid, D-/L-malic acid, glucose, fructose, sucrose, ethylene glycol, 1,2-propanediol, dihydroxyacetone, ethanol, and urea.

All substances, with the exception of sulfite, ethanol, and urea, showed no interference. In the case of sulfite, ethanol, and urea, it is recommended to dilute the solutions to 1 g/L in distilled water.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 900 mg/L glycerol (sample volume of 100 µL) with a recommended measuring range of 8 – 800 mg/L.

If this range is exceeded, the samples should be diluted with distilled water to a concentration within the measuring range. The dilution factor F must be taken into account in the calculation.

The limit of detection (LoD) was determined according to the DIN 32645:2008-11 method in a buffered aqueous solution. For a sample volume of 100 µL, the calculated LoD is 2.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 8.0 mg/L for a sample volume of 100 µL.

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* Glycerol Validation reports
- Enzytec™ *Liquid* Glycerol Excel template for results
- Enzytec™ *Liquid* Glycerol Technical Information
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

On request, we offer the following services:

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

10. Disclaimer

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