UV assay for the determination of Glucose in foodstuffs 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 evaluated using selected samples of the following matrices: wine, beer, juices, chocolate, ice cream, sweetened condensed milk, jam, molasses.

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

The test has been approved as AOAC *Official Method of Analysis* 2024.03. A publication is available in J. AOAC Int. 108(5), 677–691 (2025).

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

D-glucose is phosphorylated by the enzyme hexokinase (HK) and adenosine 5'-triphosphate (ATP) to form D-glucose 6-phosphate (G-6-P) with the simultaneous formation of adenosine 5'-diphosphate (ADP).

D-Glucose + ATP $\xrightarrow{HK}$ G-6-P + ADP

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), glucose-6-phosphate is oxidized by nicotinamide-adenine-dinucleotide (NAD) to D-gluconate-6-phosphate.

G-6-P + NAD^+ $\xrightarrow{G6P\text{-}DH}$ D-gluconate-6-P + NADH + H^+

NAD is reduced to NADH. The amount of NADH formed is stoichiometric to the amount of D-glucose and is measured at 340 nm.

The test measures free, non-covalently bound D-glucose and is suitable for differentiating between different sugars or for determining the actual concentration of various oligo- or polysaccharides, such as maltose, sucrose, lactose, or starch.

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, ATP
- Reagent 2: 2 x 12.5 mL with buffer, 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

- Sample preparation for manual and automated testing is the same.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear, colorless and almost neutral sample solutions directly or dilute sufficiently to yield a D-glucose concentration within the stated measuring range (refer to performance data).
- 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.
- 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 carbon dioxide by aid of a short ultrasound burst (e.g., 10 seconds); filter, if solution is not clear.
- 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.
- 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.

3.1. Juices & wines

- Neutralize strongly acidic samples, like juices and wines to a pH value between 6.5 and 7.5 by adding 1 M KOH. Shake or stir between additions; bring to a known volume and dilute further, if necessary, with distilled water.
- Decolorize strongly colored samples with polyvinylpolypyrrolidone (PVPP) by adding 0.1 g PVPP to 10 mL juice or wine. Stir or shake for 1 minute and filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Increase the sample volume if concentrations close to the LoQ are expected, e.g., for red wine.
- If necessary, filter turbid juices and wines; alternatively, clarify with Carrez reagents as described.

4. Manual test procedure

Wavelength:	340 nm
Temperature:	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
Measuring range:	4 – 2000 mg/L (for 100 µL sample)

	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 add:		
Reagent 2	500 µL	500 µL
Mix, incubate for **15 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 measurements** and subtracted from **each** sample result.
- Specified incubation times were validated and established 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 the D-glucose concentration

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

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

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 D-glucose is calculated using Lambert-Beer's law:

$$\mathbf{C_{D\text{-}glucose}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.744 \times \Delta A} \times F$$

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 D-glucose [g/mol]	= 180.16
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol × cm]	= 6.3 (at 340 nm)

5.2. Calculation of the D-glucose content in 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:

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

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; 0.50 g/L D-glucose).

The recovery of this multi-standard low and other aqueous control solutions should be 100 ± 5 %.

As certified reference material, we recommend, among others:

- NIST Standard Reference Material 3282, Low Calorie Cranberry Juice Cocktail (reference value: D-glucose 8.50 g/L ± 0.6 g/L, k=2)
- *Standardwein der Deutschen Weinanalytiker* (Standard wine of the German Wine Analysts; lot 1050807, reference value: D-glucose 29.57 g/L)
  https://www.weinanalytiker.de/standard-testloesung/

6. Performance data

6.1. Specificity & side activities

The test is specific for D-glucose and showed no relevant side activities with the substances examined.

Maltose only led to increased recovery at a tested concentration of 50 g/L, which can be explained by traces of D-glucose in the substance. Oligofructose and trehalose (10 g/L each) resulted in a slightly higher recovery in the presence of 0.25 g/L D-glucose.

6.2. Interferences

Related sugars and sugar alcohols such as D-sorbitol, D-mannitol, D-galactose, and lactose (50 g/L each) showed no interference in the presence of 0.376 g/L D-glucose. Psicose and tagatose (5 g/L each) showed no interference in the presence of 0.5 g/L D-glucose. D-mannose and D-fructose interfere at tested concentrations of 5.1 g/L and 12.5 g/L, respectively.

The following acids were tested for interference in the presence of 0.5 g/L D-glucose: ascorbic acid (5 g/L), L-tartaric acid, D- and L-lactic acid, acetic acid, citric acid (25 g/L each) and D-/L-malic acid (50 g/L) showed no interference. In the case of sulfite, no interference was detected at or below a concentration of 1.25 g/L. Glycerin showed no interference at 25 g/L.

Various sweeteners were tested at 10 g/L each for their interference in the presence of 0.25 g/L D-glucose: acesulfame, adventame, aspartame, cyclamate, neohesperidine, neotame, saccharin, sucralose, thaumatin, and alitame showed no interfering effect in the determination of D-glucose.

Several sugar substitutes (nutritive sweeteners) were tested at 10 g/L for their interference in the presence of 0.25 g/L D-glucose: sorbitol, mannitol, isomalt, maltitol, lactitol, xylitol, erythritol, inulin, and isomaltulose showed no interference.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 2000 mg/L D-glucose (sample volume of 100 µL) with a recommended measuring range of 4 – 2000 mg/L.

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 1.4 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 4 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 0.5 mg/L. Based on ΔA = 0.010, an LoQ of 1.0 mg/L was calculated.

6.4. Automation with Pictus 500

6.4.1. Limit of quantification (LoQ)

P500 application	LoQ
High Range	75 mg/L
Basic Range	18 mg/L
Sensitive Range	2.4 mg/L

6.4.2. Measuring ranges

P500 application	Measuring range
High Range	up to 10 g/L
Basic Range	up to 1900 mg/L
Sensitive Range	up to 190 mg/L

6.4.3. Precision and accuracy

Data from the measurement of an aqueous solution are shown here:

High Range

Target concentration, mg/L	500	1400
Mean value, mg/L	506.3	1383.8
SD, mg/L	12.62	25.22
RSD, %	2.49	1.82
Recovery, %	101	98.8

Basic Range

Target concentration, mg/L	500	1400
Mean value, mg/L	501.7	1385.2
SD, mg/L	0.88	5.96
RSD, %	0.18	0.43
Recovery, %	100	98.9

Sensitive Range

Target concentration, mg/L	50	140
Mean value, mg/L	51.5	140.7
SD, mg/L	0.71	0.42
RSD, %	1.39	0.30
Recovery, %	103	101

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* D-Glucose Validation Report
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* D-Glucose Excel template for results
- Enzytec™ *Liquid* D-Glucose Technical information
- 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

Upon request, we offer the following services, among others:

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

10. Disclaimer

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