UV assay for the determination of D-glucose and D-fructose 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: fruit and vegetable juices, soft drinks, white wine, rose wine, red wine and beer.

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

This test has been approved as AOAC *Official Method of Analysis* 2024.04 First Action. A publication is available in J. AOAC Int. 108(4), 572–594 (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 and D-fructose are phosphorylated to D-glucose-6-phosphate (G-6-P) and D-fructose-6-phosphate (F-6-P) by the enzyme hexokinase (HK) and adenosine-5'-triphosphate (ATP) with the simultaneous formation of adenosine-5'-diphosphate (ADP):

D-Glucose + ATP —— HK ⟶ D-glucose-6-phosphate + ADP

D-Fructose + ATP —— HK ⟶ D-fructose-6- phosphate + ADP

In the presence of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH), G-6-P is oxidized by nicotinamide-adenine dinucleotide (NAD) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide (NADH):

G-6-P + NAD^+ — G6P-DH → D-gluconate-6-phosphate + NADH + H^+

The amount of NADH formed during the reaction is equivalent to the amount of D-glucose and is measured at 340 nm.

Through the subsequent addition of the enzyme phosphoglucose isomerase (PGI), D-fructose-6-phosphate is also converted to D-glucose-6-phosphate, which in turn is converted to gluconate-6-phosphate and NADH:

D-Fructose-6-phosphate — PGI → D-glucose-6-phosphate

G-6-P + NAD^+ — G6P-DH → D-gluconate-6-phosphate + NADH + H^+

The amount of NADH obtained in this reaction is stoichiometric to the amount of D-fructose and is measured at 340 nm.

Note: By adding reagent 2 and reagent 3 consecutively – for example, in a fully automated system – the total amount of D-glucose and D-fructose can be determined (see 4. Manual test procedure).

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, HK, G6P-DH
- Reagent 3: 2 x 12.5 mL with buffer, PGI

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.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with distilled water to yield a total concentration of D-glucose and D-fructose not higher than 2000 mg/L (see performance data).
- Neutralize **strongly** acidic samples (e.g., wine and juices) to a pH of approx. 6.5 – 7.5 by adding 1 M KOH or adding HCl to alkaline samples.
- For turbid test samples: Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 g for at least 5 minutes) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbon dioxide by stirring in a beaker or using a short ultrasonic pulse (10 s)
- If necessary, decolorize **strongly** colored samples with polyvinylpolypyrrolidone (PVPP, e.g., 1 g/100 mL sample). Stir or shake for 1 minute and filter or centrifuge at 3000 g for at least 5 minutes until a clear supernatant is obtained.
- Grind and homogenize solid or semi-solid samples, weigh in suitable sample quantity and extract with water. Filter, centrifuge, or use Carrez clarification if necessary.
- Clarify samples containing proteins (or fat) with Carrez reagents: E.g. weigh or pipette an appropriate quantity into a 100 mL volumetric flask and add approx. 60 mL distilled water. Then add 5 mL Carrez I solution (3.60 g potassium hexacyanoferrate(II)-trihydrate $K_4[Fe(CN)_6]$ x 3 H_2O/100 mL), 5 mL Carrez II solution (7.20 g zinc sulfate $ZnSO_4$ x 7 H_2O/100 mL) and 10 mL 0.1 M NaOH. Mix well after each addition. Fill the measuring flask with distilled water up to the mark, mix and filter (discard first milliliters). 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.
- For samples with a high fat content, weigh e.g. 5 g into a 100 mL volumetric flask, fill halfway with water, and heat in a water bath at 50 – 60 °C (122 – 140 °F) for 20 minutes. After cooling, fill the flask to the mark and place it in the refrigerator for about 20 minutes to separate the fat. Then use a pleated filter to obtain a clear or slightly cloudy sample.
- Should the D-glucose/D-fructose ratio exceed e.g. 10:1, the precision of D-fructose determination might be decreased. The glucose excess must then be removed with glucose oxidase (GOD), for instance by using the Enzytec™ Glucose remover, Art. No. E3400.

4. Manual test procedure

Wavelength:	340 nm
Temperature (measurement):	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
Measuring range:	7 – 2000 mg/L for D-glucose 6 – 1000 mg/L for D-fructose

	Reagent blank	Sample / control
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)**. **Read absorbance A_2**, then add:		
Reagent 3	500 µL	500 µL
Mix, incubate for **15 minutes at 20 – 37 °C (68 – 99 °F)**. **Read absorbance A_3**.		

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 (99 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- If no differentiation between D-glucose and D-fructose is necessary, add reagent 1 and the sample solution as described and incubate for 3 minutes. Then add reagent 2 and reagent 3 consecutively (500 µL each) and incubate only once for 15 minutes at 20 – 37 °C (68 – 99 °F). Read absorbance A_2 at 340 nm and calculate the extinction difference ΔA for each cuvette.
- 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 2- or 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 relative to the creep reaction. Then, extrapolate the absorbance to the time of the last reagent addition.
- 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. Increase the sample volume if concentrations close to LoQ are expected.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary. If the sum of both sugars is higher than 2000 mg/L, the sample has to be diluted and re-analyzed.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Concentration of D-glucose

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

$$df = \frac{\text{sample volume [mL]} + \text{volume R1 [mL]}}{\text{reaction volume [mL]}} = 0.808$$

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:

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

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)

5.1.2. Concentration of D-fructose

The extinction difference ΔA must be calculated for each sample:

$$\Delta A = (A_3 - df \times A_2)_{\text{sample or control}} - (A_3 - df \times A_2)_{RB}$$

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume [mL]} + \text{vol. R1 [mL]} + \text{vol. R2 [mL]}}{\text{reaction volume [mL]}} = 0.839$$

The specified df value of **0.839 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-fructose is calculated using Lambert-Beer's law:

$$C_{\text{D-fructose}}\ [\text{g/L}] = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.887 \times \Delta A} \times F$$

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]	= 3.100
MW:	Molecular weight D-fructose [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)

5.1.3. *Total concentration of D-glucose and D-fructose without differentiation*

Perform the analysis as described in section 4.1. Important notes for assay procedure. 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

$$df = \frac{\text{sample volume [mL]} + \text{volume R1 [mL]}}{\text{reaction volume [mL]}} = 0.677$$

The specified df value of **0.677 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 the sum of D-glucose and D-fructose is calculated using Lambert-Beer's law:

$$C_{\text{D-glucose/D-fructose}}\ \mathbf{[g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.887 \times \Delta A} \times F$$

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]	= 3.100
MW:	Molecular weight D-glucose/D-fructose [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 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:

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

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, with 0.50 g/L D-glucose, and 0.50 g/L D-fructose).

The recovery of this multi-standard and other *aqueous* control solutions should be 100 ± 5 % and for extracted food samples within 100 ± 10 %.

As certified reference material, we recommend, among others:

- NIST Standard Reference Material 3282, Low Calorie Cranberry Juice Cocktail
- Standardwein der Deutschen Weinanalytiker (Standard wine of the German Wine Analysts); https://www.weinanalytiker.de/standard-testloesung/

6. Performance data

6.1. *Specificity & side activities*

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

6.2. *Interferences*

Related sugars such as sucrose, D-galactose, D-lactose and maltose (50 g/L each) showed no interference in the presence of 0.376 g/L D-glucose or 0.384 g/L D-fructose. D-mannose interferes at tested concentrations higher than 5.1 g/L.

Various concentrations of the following acids were tested for interference in the presence of 0.250 g/L D-glucose or 0.259 g/L D-fructose (or their sum): 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. Glycerol showed no interference at 25 g/L.

Several sugar substitutes (nutritive sweeteners) were tested at 10 g/L for their interference in the presence of 0.25 g/L D-glucose or D-fructose (or their sum): sorbitol, mannitol, isomalt, maltitol, lactitol, xylitol, erythritol, inulin, and isomaltulose showed no interfering effect. However, it was observed that corn starch syrup power, oligofructose, and trehalose were found to interfere the determination.

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

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 7 – 2000 mg/L.

Linearity is given up to 1000 mg/L D-fructose (sample volume of 100 µL) with a recommended measuring range of 6 – 1000 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 2.3 mg/L for D-glucose and 2.1 mg/L for D-fructose.

The limit of quantification (LoQ) was determined by precision profile. For a sample volume of 100 µL, the calculated LoQ is 6.1 mg/L for D-glucose and 5.6 mg/L for D-fructose.

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 for D-glucose and 0.57 mg/L for D-fructose. Based on ΔA = 0.010, an LoQ of 1.0 mg/L for D-glucose and 1.14 mg/L for D-fructose was calculated.

6.4. *Automation with Pictus 500*

Samples containing free D-glucose usually have to be determined separately with the Enzytec™ *Liquid* D-Glucose test (E8140) during automated processing.

The D-glucose content (E8140) obtained must be subtracted from the total result (E8160). This is because analyzers (with a few exceptions) generally have only two measuring points. Therefore, differentiation in one cuvette is not possible.

6.4.1. *Limit of quantification (LoQ)*

The measured values shown here indicate the sum of D-glucose and D-fructose.

P500 application	LoQ
High Range	75 mg/L
Basic Range	12 mg/L
Sensitive Range	5 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	**1400**	**1000**
Mean value, mg/L	1402	1020
SD, mg/L	8.49	13.11
RSD, %	0.61	1.28
Recovery, %	100	102

Basic Range

Target concentration, mg/L	**1400**	**1000**
Mean value, mg/L	1402	1016
SD, mg/L	7.08	6.93
RSD, %	0.50	0.68
Recovery, %	100	102

Sensitive Range

Target concentration, mg/L	**140**	**100**
Mean value, mg/L	140	102
SD, mg/L	0.63	0.75
RSD, %	0.45	0.74
Recovery, %	100	102

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* D-Glucose/D-Fructose Validation Report
- Enzytec™ *Liquid* D-Glucose/D-Fructose Excel template for results
- Enzytec™ *Liquid* D-Glucose/D-Fructose 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

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

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

10. Disclaimer

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d. Failure to otherwise use, and when necessary validate or verify, suitable controls, samples, matrices, or processing procedures;
e. Improper use;
f. Product alterations or modifications;
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