UV assay for the determination of sucrose/D-glucose/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: 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.

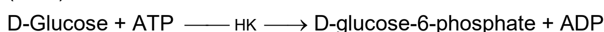
The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

1. Test principle

Sucrose is hydrolyzed with H_2O in the presence of β-fructosidase (invertase) to D-glucose and D-fructose:

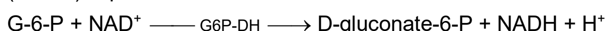
Sucrose + H_2O —— β-fructosidase ⟶ D-glucose + D-fructose

The enzyme hexokinase (HK) catalyzes the phosphorylation of D-glucose to glucose-6-phosphate by adenosine-5'-triphosphate (ATP) with the simultaneous formation of adenosine-5'-diphosphate (ADP):



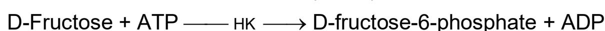
D-Glucose + ATP —— HK ⟶ D-glucose-6-phosphate + ADP

The formed D-glucose-6-phosphate (G-6-P) is specifically oxidized by nicotinamide adenine dinucleotide phosphate (NAD) in the presence of glucose-6-phosphate dehydrogenase (G6P-DH) to D-gluconate-6-phosphate, yielding reduced nicotinamide adenine dinucleotide (NADH) is produced:



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

Hexokinase also catalyzes the phosphorylation of D-fructose by ATP to form D-fructose-6-phosphate (F-6-P):



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

Following this reaction, F-6-P is also converted to G-6-P by the enzyme phosphoglucose isomerase (PGI):

D-Fructose-6-Phosphat —— PGI ⟶ D-glucose-6-phosphate

G-6-P then reacts with NAD to form D-gluconate-6-phosphate and NADH. The amount of NADH formed in all the subreactions is equivalent to the total amount of sucrose, D-glucose, and D-fructose and is measured at 340 nm.

The test Enzytec™ *Liquid* Sucrose/D-Glucose/D-Fructose E8190 is suitable for determining the sum of sucrose, (free) D-glucose and D-fructose ("total sugar").

To determine the three sugar types individually, the Enzytec™ *Liquid* D-Glucose/D-Fructose E8160 test can be used. The (actual) sucrose content is then calculated by subtracting the D-glucose and D-fructose concentrations.

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, invertase, PGI 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

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 a concentration within the measuring range (see performance data).
- Neutralize **strongly** acidic samples to a pH of approx. 8.0 by adding 1 M KOH or NaOH 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 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbon dioxide (e.g., beer) 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 rpm 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/sucrose ratio exceed e.g. 10:1, the precision of sucrose determination decreases. This scenario can be observed in honey, for instance, where sucrose levels are usually minimal and D-glucose levels are notably high. The glucose excess must then be removed with GOD (e.g., using Enzytec™ Glucose remover).

3.2. Juices & wines

- Neutralize strongly acidic juices and wines. 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.

3.3. Chocolate

- For samples with a high fat content like chocolate: weigh a sufficient sample quantity (finely grated) into a volumetric flask.
- Extract with hot water (approximately 70 °C/158 °F) and heat in a water bath at 60 – 65 °C (140 – 149 °F) for 20 minutes.
- Cool down for fat separation, fill up to the mark with water (fat above the mark).
- Place the volumetric flask in an ice bath for 15 minutes and filter (fluted paper filter or syringe filter).
- Use a clear filtrate for analysis.
- Alternatively, clarify with Carrez reagents as described above.

3.4. Further application examples (not validated)

3.4.1. Sweetened condensed milk and ice cream

- Weigh approx. 1 g sample accurately into a 100 mL volumetric flask.
- Add approx. 60 ml water and incubate for 15 minutes at approx. 70 °C (158 °F); stir occasionally.
- Apply Carrez clarification as described above.
- If necessary, dilute the sample according to the specified measuring range, and use a clear solution for analysis.

3.4.2. Honey

- Take approximately 10 g of viscous (or crystalline) honey and heat it in a beaker for 15 minutes at approximately 60°C, stirring occasionally with a spatula (there is no need to heat liquid honey). Allow to cool.
- Accurately weigh approximately 1 g of the liquid sample into a 100 ml volumetric flask. At first, dissolve with only a small amount of water. Then fill up to the mark.
- If the estimated sucrose content in the honey sample is around 5 – 10 %, the 1 % solution should be diluted in a ratio of, for example, 1:3 before analysis.
- If the estimated sucrose content in the honey sample is very low (e.g., 0.5 – 5 %), the excess D-glucose should be removed with GOD (Enzytec™ Glucose Remover).

4. Manual test procedure

Wavelength: 340 nm
Temperature (measurement): 20 – 37 °C (68 – 99 °F)
Photometer alignment: against air (without cuvette)
Measuring range: 10 – 2000 mg/L (for 100 µL)

	Reagent blank	Samples / controls
Reagent 1 **Sample / control** **Dist. water**	2000 µL - 100 µL	2000 µL 100 µL -
Mix, incubate for **15 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 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)**.
- 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 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 total sugar (sum of sucrose, D-glucose and D-fructose)

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 total sugar is calculated using Lambert-Beer's law:

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

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

The result includes the amount of sucrose, D-glucose and D-fructose which are present in the sample. It is calculated as "total sugar", with the molecular weight of D-glucose (180.16 g/mol).

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

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

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, with 0,50 g/L sucrose, 0,50 g/L D-glucose, 0,50 g/L D-fructose).

To obtain the target concentration, the ratio between the molecular weights of the two sugars must be taken into account, for example:

MW_{sucrose} 342,3 g/mol : $MW_{\text{D-glucose}}$ 180,16 g/mol → factor 1.9

$$\text{Total sugar} = (C_{\text{sucrose}} \div 1.9) \times 2 + C_{\text{D-Glucose}} + C_{\text{D-Fructose}}$$
$$= (0.50\ \text{g/L} \div 1.9) \times 2 + 0.50\ \text{g/L} + 0.50\ \text{g/L} = \mathbf{1.526\ g/L}$$

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

As certified reference material, we recommend, among others:

- Fortified Breakfast Cereals; NIST SRM 3233
- Chocolate confectionery; LGC 7016

6. Performance data

6.1. Specificity & side activities

The test is specific to sucrose and D-glucose. A slight side activity towards oligosaccharides of the raffinose type, e.g. L-raffinose is given, however, hydrolysis proceeds much more slowly. Any creep reactions that occur can be eliminated by mathematical extrapolation.

6.2. Interferences

Several sugar substitutes (nutritive sweeteners) have been tested with 10 g/L in a multi-sugar standard (containing sucrose, glucose and fructose with a concentration of 0.763 g/L total sugar) during the validation for interference: sorbitol, mannitol, isomalt, maltitol, lactitol, xylitol, erythritol, inulin, isomaltulose and trehalose. All tested substances showed no interference.

Various sweeteners have been tested for interference in the presence of the 0.763 g/L multi-sugar standard with 2 g/L or 5 g/L: acesulfame, adventame, aspartame, cyclamate, neohesperidin, neotame, saccharin, sucralose, thaumatin and alitame showed no interfering effect in the determination.

Additionally the following substances were tested with a concentration of 1 g/L in 0.763 g/L multi-sugar standard: Ascorbic acid, SO_2, D-/L-lactic acid, acetic acid, D-/L-tartaric acid, citric acid, D-/L-malic acid, NaCl, taurine, glycerin, oxalic acid, benzoic acid, gluconic acid, mucic acid and glucosamine. All tested substances showed no interference. In case of sulfite, a dilution in dist. Water ≤ 0.1 g/L is recommended.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 2000 mg/L total sugar (sample volume of 100 µL) with a recommended measuring range between 10 and 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 6.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 10.0 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 0.5 mg/L. Based on ΔA = 0.010, an LoQ of 1.0 mg/L was calculated.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Sucrose/D-Glucose/D-Fructose Validation Report
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Sucrose/D-Glucose/D-Fructose Excel template for results
- Enzytec™ *Liquid* Sucrose/D-Glucose/D-Fructose 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

This information represents our present understanding and is meant to inform you about our products and their potential uses. It is not a guarantee of particular qualities or suitability for any specific purpose.

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b. Failure to use trained and qualified personnel;
c. Failure to apply appropriate industry standard practices, including Good Laboratory Practices;
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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