UV assay for the determination of Sucrose/D-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 testkit was approved as AOAC *Official Method of Analysis* 2024.05. This also covers the tests Enzytec™ *Liquid* D-Glucose E8140 and Enzytec™ *Liquid* Combi Sucrose/D-Glucose E8185. A publication is available in J. AOAC Int. 108:151–172 (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

The enzymatic reaction requires three enzymes (β-fructosidase, hexokinase, glucose-6-phosphate-dehydrogenase) and the co-enzyme nicotinamide-adenine-dinucleotide (NAD). The D-glucose concentration is determined after inversion (total D-glucose).

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-triphosphate (ATP) with the simultaneous formation of adenosine-diphosphate (ADP):

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

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

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

The amount of resulting NADH is stoichiometric to the amount of sucrose and free D-glucose and is measured at 340 nm.

The test Enzytec™ Liquid Sucrose/D-Glucose (E8180) is primarily suitable for determining the sum of sucrose and free D-glucose. For individual determination of D-glucose (before inversion), use Enzytec™ Liquid D-Glucose E8140. The (real) sucrose content is then calculated from the difference of the D-glucose concentrations before and after enzymatic inversion.

## 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, β-fructosidase, 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 sucrose/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.
- 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).

### *3.1. 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.

### *3.2. 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.

### *3.3.* Further application examples (not AOAC-validated)

#### *3.3.1. 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.

#### *3.3.2. 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: | 20 – 37 °C (68 – 99 °F) |
| Photometer alignment: | against air (without cuvette) |
| Measuring range: | 10 – 2500 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 **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 once for each test series** and subtracted from **every** 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 sample solutions*

#### *5.1.1. Concentration of total sucrose (sum of sucrose and free D-glucose)*

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 sucrose is calculated using Lambert-Beer's law:

$$\mathbf{C}_{\text{total sucrose}}\ \mathbf{[g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{1.412 \times \Delta A} \times F$$

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 sucrose [g/mol] | = 342.3 |
| 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. Calculation of the real sucrose concentration after differentiation of sucrose and D-glucose*

##### *5.1.2.1. Differentiation of sucrose und D-glucose*

To distinguish the individual types of sugar, free D-glucose must be determined separately using Enzytec™ *Liquid* D-Glucose E8140 and then subtract this result from the total sucrose obtained with Enzytec™ *Liquid* Sucrose/D-Glucose E8180. The ratio between the molecular weights of the two sugars must be taken into account:

$MW_{sucrose}$ 342.3 g/mol : $MW_{D\text{-glucose}}$ 180.16 g/mol → factor 1.9

$$C_{sucrose}\ [g/L] = C_{total\ sucrose\ E8180} - 1.9 \times C_{D\text{-glucose E8140}}$$

For further information on assay performance of Enzytec™ *Liquid* D-Glucose E8140, please refer to the corresponding package insert.

**Example:**

| | | | | |
|---|---|---|---|---|
| Total sucrose (E8180) | | | = | 1.500 g/L |
| D-Glucose (E8140) | | | = | 0.400 g/L |
| Real Sucrose | = | 1.500 g/L - 1.9 × 0.400 g/L | = | **0.740 g/L** |

### *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:

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

### *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; 0.50 g/L sucrose and D-glucose each).

$$\text{Total sucrose} = (0.50\ \text{g/L} \times 1.9)_{\text{D-glucose}} + 0.50\ \text{g/L}_{\text{sucrose}} = 1.45\ \text{g/L}$$

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: c = 13.42 g/100 g sucrose, 0.81 g/100 g fructose, 1.04 g/100 g glucose
- Chocolate confectionery; LGC 7016: c = 46.5 g/100 g sucrose (Lot 003)

## 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.

### *6.2. Interferences*

Several sugar substitutes (nutritive sweeteners) have been tested for interference in the presence of sucrose (0.25 g/L) and D-glucose (0.25 g/L): sorbitol, mannitol, isomalt, maltitol, lactitol, xylitol, erythritol, inulin, isomaltulose showed no interfering effect on the determination, whereas oligofructose and trehalose lead to overestimation.

Various sweeteners have been tested for interference in the presence of sucrose (0.25 g/L) and D-glucose (0.25 g/L): acesulfame, adventame, aspartame, cyclamate, neohesperidin, neotame, saccharin, sucralose, thaumatin and alitame showed no interfering effect in the determination of sucrose/D-glucose.

Raffinose interferes at concentrations higher than 8.34 g/L, while mannose and fructose react at concentrations higher than 6.25 g/L and 12.5 g/L, respectively.

The following acids were tested in the presence of sucrose (0.25 g/L) and D-glucose (0.25 g/L): D-/L-lactic acid, D-/L-malic acid, D-/L-tartaric acid, citric acid, acetic acid, oxalic acid, gluconic acid and galactaric acid and benzoic acid at levels of 10 g/L or less. No interference was detected in the presence of these substances.

For sulphite, an increased recovery was determined. This effect was no longer observed at 0.5 g/L sulphite or lower. NaCl, taurin, glycerol and glucosamine showed no interferences.

### *6.3. Linearity, measuring range & sensitivity*

Linearity is given up to 3000 mg/L total sucrose (sample volume of 100 µL) with a recommended measuring range between 10 and 2500 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 5 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 10 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 0.95 mg/L. Based on ΔA = 0.010, an LoQ of 1.9 mg/L was calculated.

### *6.4. Automation with Pictus 500*

For the differentiation of sucrose and D-glucose, see chapter 5.1.2.

#### *6.4.1. Limit of quantification (LoQ)*

| P500 application | LoQ |
|---|---|
| High Range | 75 mg/L |
| Basic Range | 15 mg/L |
| Sensitive Range | 3.8 mg/L |

#### *6.4.2. Measuring ranges*

| P500 application | Measuring range |
|---|---|
| High Range | up to 9.5 g/L |
| Basic Range | up to 1.9 g/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 | 1500 | 1450 |
|---|---|---|
| Mean value, mg/L | 1511 | 1499 |
| SD, mg/L | 8.33 | 8.60 |
| RSD, % | 0.55 | 0.57 |
| Recovery, % | 100.8 | 103.4 |

***Basic Range***

| Target concentration, mg/L | 1500 | 1450 |
|---|---|---|
| Mean value, mg/L | 1540 | 1501 |
| SD, mg/L | 4.32 | 5.33 |
| RSD, % | 0.28 | 0.36 |
| Recovery, % | 102.7 | 103.5 |

***Sensitive Range***

| Target concentration, mg/L | 150 | 145 |
|---|---|---|
| Mean value, mg/L | 152.4 | 1.55 |
| SD, mg/L | 0.75 | 1.55 |
| RSD, % | 0.49 | 1.03 |
| Recovery, % | 101.6 | 103.8 |

## 7. Supporting documents

On request, we offer the following documents:

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