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

1. Test principle

The enzymatic reaction requires three enzymes (ß-fructosidase, hexokinase, glucose-6-phosphate-dehydrogenase) and one co-enzyme (NAD). The reaction takes place in four steps:

Sucrose is hydrolysates with H_2O, through the presence of ß-fructosidase, to D-glucose + D-fructose:

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

The resulting D-glucose is phosphorylated in the presents of ATP and a hexokinase (HK) to glucose-6-phosphate:

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

The glucose-6-phosphate-dehydrogenase (G6P-DH) oxidizes the formed glucose-6-phosphate with NAD^+ to gluconate-6-phosphate + NADH + H^+:

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

Nicotinamide-adenine-dinucleotide (NAD) is reduced to NADH. The amount of resulting NADH is equivalent to the converted amount of sucrose and free D-glucose 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, ß-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) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- Weigh samples with a high fat content into a volumetric flask and extract with hot water; allow sample solution to cool down for fat separation (e.g. 15 min in an ice bath); fill volumetric flask up to the mark with water, filter aqueous solution before testing.
- Clarify samples containing proteins or fat with Carrez clarification.
- Crush and homogenize solid or semi-solid samples and extract with water; filtrate or centrifuge, or use Carrez clarification if necessary.
- If necessary, adjust the pH to 7 by adding KOH / NaOH to acidic samples, or by adding HCl to alkaline samples.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 10 - 2500 mg/L

	Reagent blank	Samples / controls
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 15 min at 20 - 37 °C. Read absorbance A_1, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate for 15 min at 20 - 37 °C and read absorbance A_2.		

The reagent blank value must be determined once for each run and subtracted from each sample result.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of sucrose (sucrose + D-glucose)

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

df: Dilution factor
RB: Reagent blank

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

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume of very acidic samples is increased, the test system may be affected. This must be checked.

$$\mathbf{C_{total\ sucrose}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{1.413 \times \Delta A}$$

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 342.3
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)

5.1.2. Calculation of the sucrose concentration after differentiation of sucrose and D-glucose

5.1.2.1. Differentiation of sucrose und D-glucose

If differentiation of both types of sugar is preferred, the free D-glucose must also be determined separately using the Enzytec™ *Liquid* D-Glucose test (E8140) test and subtracted from the result of the Enzytec™ *Liquid* Sucrose/D-Glucose (E8180) test. The ratio between the molecular weights of the two sugars must be taken into account ($MW_{sucrose}$ 342.3 g/mol and $MW_{D\text{-}glucose}$ 180.16 g/mol).
For further information, please refer to the corresponding package inserts for D-Glucose (E8140) and Sucrose/D-Glucose (E8180).

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

Example: Enzytec™ *Liquid* Multi-Sugar Standard low E8440

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

If the D-glucose / sucrose ratio is higher than 10:1, the precision of the sucrose determination decreases. In this case, the glucose excess must be eliminated with the Glucose Remover (E3400).

5.2. Calculation of solid samples

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

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. For this purpose, we recommend the use of Enzytec™ *Liquid* Multi-Sugar Standard low (E8440).
The recovery of Enzytec™ *Liquid* Multi-Sugar Standard low and other aqueous control solutions should be within 100 ± 5 % and for extracted food samples within 100 ± 10 %.

6. Performance data

6.1. Specificity & side activities

The test is specific for sucrose and D-glucose. Oligosaccharides of the raffinose type will hydrolyzed, but more slowly than sucrose.
The Enzytec™ *Liquid* Sucrose/D-Glucose system shows a slight side activity towards oligosaccharides of the raffinose type, e.g. L-raffinose.
Sample solutions containing free D-glucose must be tested separately with the Enzytec™ *Liquid* D-Glucose (E8140) test. The sugar content obtained must be subtracted from the total result as described in section *Calculation of results* to distinguish the individual types of sugar.

6.2. Interferences

The test shows no interferences to different relevant alcohols, acids, sweeteners and most of the sugars. In case of sulfite, a dilution in dist. water ≤ 0.5 g/L is recommended.

6.3. Linearity, measuring range & sensitivity

Linearity is given at 2 to 3000 mg/L total sucrose, with the recommended measuring range between 10 und 2500 mg/L (sample volume of 100 µL).
The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11 in buffered aqueous solution. This results in an LoD of 5.0 mg/L. The limit of quantification (LoQ) was determined by precision profile and is 10.0 mg/L.
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 5.1.2.1.

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	to 9.5 g/L
Basic Range	to 1.9 g/L
Sensitive Range	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* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

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

9. Disclaimer

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