

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

1. Test principle

Enzymatic determination of the sum of sucrose, D-glucose and D-fructose ("total sugar") with β-fructosidase (invertase), hexokinase (HK), phosphoglucose isomerase (PGI) and glucose-6-phosphate-dehydrogenase (G6P-DH).

Sucrose + H₂O $\xrightarrow{\beta\text{-fructosidase}}$ D-glucose + D-fructose

D-Fructose + ATP $\xrightarrow{\text{HK}}$ fructose-6-phosphate + ADP

D-Glucose + ATP $\xrightarrow{\text{HK}}$ glucose-6-phosphate + ADP

Fructose-6-phosphate $\xrightarrow{\text{PGI}}$ glucose-6-phosphate

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

Nicotinamide-adenine-dinucleotide (NAD) is reduced to NADH. The amount of NADH formed is proportional to the amount of sucrose, D-glucose and D-fructose 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, β-fructosidase, ATP, PGI
- 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.
- 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.
- 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.

4. Assays performance

Wavelength: 340 nm
 Temperature: 20 - 37 °C (during the measurement)
 Measurement: against air (without cuvette) or water
 Measuring range: 10 - 2000 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 addition of:		
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. Concentration of total sugar (sucrose, D-glucose, D-fructose)

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

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 is increased, the test system may be affected. In general, this must be checked depending on the matrix.

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

V: Test volume basic application [mL] = 2.600
 MW: Molecular weight [g/mol] = 180.16
 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)

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).

5.2. Calculation of solid samples

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

5.3. Controls & acceptance criteria

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 %.

6. Performance data

6.1. Specificity

The test is specific for sucrose, D-glucose and D-fructose.

6.2. Interferences & side activities

The test shows no interferences to different relevant alcohols, acids, sweeteners and most of the sugars. Oligosaccharides of the raffinose type can lead to a creep reaction, which can be eliminated by mathematical extrapolation.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 2000 mg/L total sugar (sample volume of 100 µL).

The limit of detection (LoD) was determined for a sample volume of $v = 100 \mu\text{L}$ according to method DIN 32645:2008-11, using buffered aqueous solutions. This results in an LoD of 6 mg/L.

The limit of quantification (LoQ) was determined by precision profile and is 10 mg/L.

The smallest absorbance difference that the method can distinguish is $\Delta A = 0.005$. For a sample volume of $v = 1000 \mu\text{L}$, this results in an LoD of 0.5 mg/L. Based on $\Delta A = 0.010$, an LoQ of 1 mg/L was calculated.

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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