Enzymatic assay for foodstuff and other sample materials
2 x 50 ml R1 / 2 x 12.5 ml R2 (50 assays)

For *in vitro* use only
Store between +2 and +8°C

Principle

Enzymatic test with ß-Fructosidase (Invertase), Hexokinase (HK) and Glucose-6-Phosphate Dehydrogenase (G6P-DH). NADH is produced and is measured at 340 nm:

Sucrose + H_2O —— ß-Fructosidase ⟶ D-Glucose + D-Fructose
D-Glucose + ATP —— HK ⟶ Glucose-6-Phosphate + ADP
G-6-P + NAD^+ —— G6P-DH ⟶ Gluconate-6-P + NADH + H^+

Reagents

The reagents are ready-to-use.

Reagent 1: two vials ≥ 50 ml (NAD, ß-Fructosidase, ATP)

Reagent 2: two vials ≥ 12.5 ml (HK, G6P-DH)

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C. Do not freeze the reagents. Let the reagents reach the laboratory temperature before use (20 - 25 °C).

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 material safety data sheets (MSDS) for this product, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Sample preparation

- Use liquid and clear samples directly, or after dilution into the relevant measuring range (see test performance)
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide
- 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
- For fat containing samples, weigh sample into a volumetric flask (min. 50 ml) and extract with hot water; cool to allow the fat to separate; make up to the mark with water, remove the fatty layer on the top and filter the aqueous part
- Adjust the pH to approx. 7.0 by adding KOH / NaOH to acidic samples, or by adding HCl to alkaline samples

Assay procedure

Wavelength:	340 nm
Optical path:	1 cm
Temperature:	20 – 25 °C / 37 °C
Measurement:	Against air or against water
Sample solution:	20 – 1500 mg/l

	Reagent Blank (RB)	Samples
Sample / Standard	-	100 µl
Dist. water	100 µl	-
Reagent 1	2000 µl	2000 µl
Mix, incubate for approx. 15 min. at 20 – 25 °C. Read absorbance A1, then add:		
Reagent 2	500 µl	500 µl
Mix, wait until the end of the reaction (approx. 15 min. at 20 - 25 °C). Read absorbance A2.		

Reagent blank must be performed once for every run, and subtracted from every sample during calculation of results.

Calculation of results

Total-sucrose (Sucrose + free D-Glucose)

$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$
With df = dilution factor of optical densities, because of reagent volumes:
df = (sample + R1) / (sample + R1 + R2) = 0.808.

c = (V x MW x Δ A) / (ε x d x v x 1000) [in g/l of Total-sucrose]
c = (2.600 x 342.30 x Δ A) / (ε x 1 x 0.1 x 1000)
It results for the determination at 340 nm (ε = 6.3 l x $mmol^{-1}$ x cm^{-1}):

$c_{Total\ Sucrose}$ [g/l] = **1.413** x ΔA

Calculation of Real Sucrose

The result above includes the amount of Sucrose plus the free D-Glucose which is present in the sample. It is calculated as "Total Sucrose", with the molecular weight of Sucrose (342.3 g/mol). For differentiation of the two sugars, the free D-Glucose must be measured with the Enzytec™ *Liquid* D-Glucose assay (E8140) in a separate run. Sucrose is calculated by subtraction of the free D-Glucose content from the Total-sucrose content. For this calculation, the ratio between the molecular weights of both sugars must be considered (factor 1.9):

$$C_{Sucrose}\ [g/l] = C_{Total\text{-}sucrose} - 1.90 \times C_{Glucose}$$

Example:

Total-sucrose (E8180)	*1.500 g/l*
D-Glucose (E8140)	*0.400 g/l*
Sucrose = 1.500 g/l – 1.90 x 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).

Calculation in solid samples

$$\text{Content [g/100 g]} = \frac{C_{Analyte}\ [g/l]}{weight_{sample}\ [g/l]} \times 100$$

Test performance

Specificity

The test is specific for Sucrose and D-Glucose. Oligosaccharides of the Raffinose type will hydrolyzed, but more slowly than sucrose.

Measuring range

The recommended measuring range is 20 - 1500 mg/l (Sucrose and D-Glucose). When values exceed this range, samples should be diluted into the range 100 to 1500 mg/l.

Sensitivity

The Limit of Detection (LoD) and Limit of Quantification (LoQ) where determined according to the method DIN 32645:2008-11:

- LoD = 10 mg/l (Total Sucrose)
- LoQ = 16 mg/l (Total Sucrose)

Automation

Application sheets for automated systems are available on request.

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