Determination of Sucrose, D-Glucose and D-Fructose in food products (without differentiation)
Test-kit for 32 determinations on the RIDA®CUBE SCAN instrument (340 nm)

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

Principle

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

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

Reagents

1: 32 tubes with 800 µl reagent 1 (NAD, ß-Fructosidase)
2: 32 caps with 200 µl reagent 2 (HK / PGI / G6P-DH)
3: one RFID card (Radio Frequency Identification)

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), 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 clear, colourless and pH-neutral liquid samples directly, or after dilution into the relevant measuring range
- 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 (for example on an ice bath for 15 min); make up to the mark with water, remove the fatty layer on the top and filter the aqueous part before testing
- Adjust the pH to approx. 5.0 by adding KOH / NaOH to acidic samples or by adding HCl to alkaline samples

Assay specifications

The assay specifications are saved on the RFID card and are executed automatically by the instrument.

Wavelength:	340 nm
Temperature:	37 °C
Calibration:	calibration curve saved on RFID card
Test sequence:	Sample + R1 / mix / 5 min / A1 / R2 / mix / 10 min / A2
Sample volume:	20 µl (basic) or 100 µl (sensitive). The selected volume must be pipeted precisely into reagent 1 (test tube).

Handling procedure

Place the RFID Card on the instrument	
Enter sample data into tablet app: - identification - volume (20 or 100 µl)	
Pipette the sample into the test-tube (reagent 1)	 
Close the tube with the cap (reagent 2), insert it into the instrument and close the door	 

Calculation of results

The result includes the amount of Sucrose, D-Glucose and D-Fructose which are present in the sample (without differentiation).

The results are given in mg/l by the instrument, and following ranges are recommended:
- from 100 to 2200 mg/l for the basic application (20 µl)
- from 20 to 450 mg/l for the sensitive application (100 µl)

The sample volume is 20 µl or 100 µl. For the sensitive application, it is also possible to pipette any dilution with 100 µl total volume (for example 50 µl sample and 50 µl water). Results must be recalculated accordingly.

Notes

1. The test is specific for Sucrose, D-Glucose and D-Fructose. Oligosaccharides of the Raffinose type will be hydrolyzed, but more slowly than sucrose.
2. Use a quality control every day where a run is performed (e.g. Enzytec Fluid sugar standard E5440). If the deviation of this quality control is higher than 10%, it is recommended to measure the reagent blank with a water sample, and to subtract it from all future sample results.