Enzymatic assay for foodstuff and other sample materials
2 x 50 ml R1 + 2 x 12.5 ml R2 + 2 x 12.5 ml R3 (50 assays)

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

Principle

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

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

The reagents are ready-to-use:
#1: Reagent 1, approx. 50 ml x 2 vials (NAD, buffer)
#2: Reagent 2, approx. 12.5 ml x 2 vials (HK, G6P-DH)
#3: Reagent 3, approx. 12.5 ml x 2 vials (PGI)

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 further 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 clear, colourless and pH-neutral liquid 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. 8.0 by adding KOH / NaOH to acidic samples or by adding HCl to alkaline samples

Assay procedure

Wavelength: 340 nm, Hg 334 nm, Hg 365 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 1 min. at 37 °C or 3 min. at 20 - 25 °C. Read absorbance A1, then add:		
Reagent 2	500 µl	500 µl
Mix, wait until the end of the reaction (incubation for approx**. 10 min. at 37°C** or approx. 15 min. at 20 - 25 °C). Read absorbance A2, then add:		
Reagent 3	500 µl	500 µl
Mix, wait till the end of the reaction (incubation for approx. **10 min. at 37°C** or approx. 15 min. at 20 - 25°C). Read absorbance A3.		

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

Calculation of results

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 volume + R1) / (sample volume + R1 + R2) = 0.808.

c = (V x MW x Δ A) / (ε x d x v x 1000) [in g/l of D-glucose]
c = (2.600 x 180.16 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}):

$c_{D\text{-}Glucose}$ [g/l] = **0.744** x ΔA

D-Fructose

$\Delta A = (A_3 - df \times A_2)_{sample} - (A_3 - df \times A_2)_{RB}$
With df = dilution factor of optical densities, because of reagent volumes:
df = (sample volume+R1+R2) / (sample volume+R1+R2+R3) = 0.839.

c = (V x MW x Δ A) / (ε x d x v x 1000) [in g/l of D-Fructose]
c = (3.100 x 180.16 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}):

$c_{D\text{-}Fructose}$ [g/l] = **0.887** x ΔA

D-Glucose and D-Fructose without differentiation

Add reagent 2 and reagent 3 simultaneously and incubate only once.

$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$
With df = dilution factor of optical densities, because of reagent volumes:
df = (sample volume + R1) / (sample volume + R1 + R2 + R3) = 0.677.

c = (V x MW x Δ A) / (ε x d x v x 1000) [g/l of D-glucose/D-Fructose]
c = (3.100 x 180.16 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}):

$c_{D\text{-}Glucose\ /\ D\text{-}Fructose}$ [g/l] = **0.887** x ΔA

Calculation in solid samples

$$\text{Content [g/100 g]} = \frac{c_{D\text{-}Glucose\ /\ D\text{-}Fructose}\ [g/l]}{weight_{sample}\ [g/l]} \times 100$$

Test performance

Specificity

The test is specific for D-Glucose and D-Fructose. No interference was observed for Galactose, Lactose, Maltose, Mannitol, Sorbitol and Sucrose. Mannose shows no interferences up to 5 g/l but leads to low recoveries at higher concentrations. If the D-Glucose/ D-Fructose ratio is higher than 10:1, the precision of the D-Fructose determination decreases.

Measuring range

The test determines Glucose and Fructose concentrations from 20 to 1500 mg/l (total for the two sugars). When values exceed this range, samples should be diluted into the range 100 – 1500 mg/l with dist. water. The dilution factor has to be considered in the calculation.

Sensitivity

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

- LoD = 4.0 mg/l
- LoQ = 10 mg/l

Automation

Application sheets for automated systems are available on request.

Disclaimer

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