UV assay for the determination of 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 test with hexokinase (HK), phosphoglucose-isomerase (PGI) and glucose-6-phosphate-dehydrogenase (G6P-DH).

D-Glucose and D-fructose are phosphorylated by the enzyme hexokinase (HK) and ATP to glucose-6-phosphate (G6P) or fructose-6-phosphate (F6P).

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

D-Fructose + ATP — HK → fructose-6-phosphate + ADP

In the presence of the enzyme glucose-6-phosphate-dehydrogenase (G6P-DH), G6P is oxidized by nicotinamide-adenine-dinucleotide (NAD^+) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide (NADH).

Addition of phosphoglucose-isomerase (PGI) convert fructose-6-phosphate (F6P) to glucose-6-phosphate (G6P) which in turn is converted to gluconate-6-phosphate and NADH by G6P-DH.

Fructose-6-phosphate — PGI → glucose-6-phosphate

G6P + NAD^+ — G6P-DH → gluconat-6-P + NADH + H^+

Nicotinamide-adenine-dinucleotide (NAD) is reduced to NADH. The amount of NADH formed is proportional to the amount of D-glucose/ D-fructose formed 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
- Reagent 2: 2 x 12.5 mL with buffer, HK, G6P-DH
- Reagent 3: 2 x 12.5 mL with buffer, PGI

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: 7 - 2000 mg/L for D-glucose
6 - 1000 mg/L for D-fructose

	Reagent blank	Sample / control
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 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.		
Reagent 3	500 µL	500 µL
Mix, incubate for 15 min at 20 - 37 °C and read absorbance A_3.		

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 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.

$$\mathbf{C_{total\ D\text{-}glucose}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.744 \times \Delta A}$$

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)

5.1.2. Total concentration of D-fructose

$$\Delta A = (A_3 - df \times A_2)_{sample} - (A_3 - df \times A_2)_{RB}$$

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume} + R1 + R2}{\text{test volume}} = 0.839$$

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.

$$\mathbf{C_{total\ D\text{-}fructose}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.887 \times \Delta A}$$

V:	Test volume basic application [mL]	= 3.100
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)

5.1.3. D-glucose and D-fructose without differentiation

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

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume} + R1}{\text{test volume}} = 0.677$$

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.

$$\mathbf{C_{total\ D\text{-}glucose/D\text{-}fructose}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.887 \times \Delta A}$$

V:	Test volume basic application [mL]	= 3.100
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)

5.2. Calculation of solid samples

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

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

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

6.2. Interferences & side activities

A total of over 35 different substances were tested for interference from the following three categories: related sugars, important acids, glycerol and sulfite, sweeteners and sugar substitutes. Mannose showed no interference below 5.1 g/L. Sulfite interferes at 1.25 g/L or higher.
The Enzytec™ *Liquid* D-Glucose/D-Fructose assay shows no side activity to relevant sugars or sugar alcohols.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 2000 mg/L D-glucose, with the recommended measuring range between 7 and 2000 mg/L (sample volume of 100 µL).
Linearity is given up to 1000 mg/L D-fructose, with the recommended measuring range between 6 and 1000 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, using aqueous control solutions. This results in an LoD of 2.3 mg/L for D-glucose and 2.1 mg/L for D-fructose. The limit of quantification (LoQ) was determined by precision profile and is 6.1 mg/L for D-glucose and 5.6 mg/L for D-fructose.
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 0.5 mg/L for D-glucose and 0.57 mg/L for D-fructose. Based on ΔA = 0.010, an LoQ of 1 mg/L for D-glucose and 1.14 mg/L for D-fructose was calculated.

6.4. Automation with Pictus 500

Samples containing free D-glucose usually have to be determined separately with the Enzytec™ *Liquid* D-Glucose test (E8140) during automated processing. The D-glucose content (E8140) obtained must be subtracted from the total result (E8160). This is because analyzers (with a few exceptions) generally have only two measuring points. Therefore, differentiation in one cuvette is not possible.

6.4.1. Limit of quantification (LoQ)

The measured values shown here indicate the sum of D-glucose and D-fructose.

P500 application	LoQ
High Range	75 mg/L
Basic Range	12 mg/L
Sensitive Range	5 mg/L

6.4.2. Measuring ranges

P500 application	Measuring range
High Range	to 10 g/L
Basic Range	to 1900 mg/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	1400	1000
Mean value, mg/L	1402	1020
SD, mg/L	8.49	13.11
RSD, %	0.61	1.28
Recovery, %	100	102

Basic Range

Target concentration, mg/L	1400	1000
Mean value, mg/L	1402	1016
SD, mg/L	7.08	6.93
RSD, %	0.50	0.68
Recovery, %	100	102

Sensitive Range

Target concentration, mg/L	140	100
Mean value, mg/L	140	102
SD, mg/L	0.63	0.75
RSD, %	0.45	0.74
Recovery, %	100	102

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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