UV assay for the determination of maltose, sucrose and D-glucose in foodstuffs and other sample materials
Test combination for 50 determinations

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

1. Test principle

The enzyme α-glucosidase (maltase) cleaves maltose and sucrose into two molecules D-glucose and D-glucose and D-fructose, respectively. Furthermore, sucrose is also hydrolyzed to D-glucose and D-fructose by the enzyme β-fructosidase (invertase):

Maltose + H_2O —α-glucosidase→ 2 D-glucose

Sucrose + H_2O —α-glucosidase→ D-glucose + D-fructose

Sucrose + H_2O —β-fructosidase→ D-glucose + D-fructose

D-glucose is phosphorylated with ATP in the presence of the enzyme hexokinase (HK) to D-glucose-6-phosphate (G-6-P), simultaneously producing ADP:

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

In the presence of a glucose-6-phosphate dehydrogenase (G6P-DH), D-glucose-6-phosphate is oxidized to D-gluconate-6-phosphate:

G-6-P + NAD^+ —G6P-DH→ D-gluconate-6-phosphate + NADH + H^+

In this process, nicotinamide adenine dinucleotide (NAD) is reduced to NADH. The amount of NADH formed is proportional to the amount of D-glucose formed and is measured at 340 nm. The result is expressed as total maltose [g/L].

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, maltase, invertase, NAD, ATP
- 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, available online at www.r-biopharm.com. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water. Water to a concentration within the measuring range (see performance data).
- Filter or centrifuge turbid solutions.
- Degas samples containing carbonic acid.
- Grind and homogenize solid or semi-solid samples, weigh in suitable sample quantity and extract with water. If necessary, centrifuge, filter or perform Carrèz clarification.
- 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
Sample: 10 - 1100 mg/L

	Reagent blank	Sample / control
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate approx. 30 min at 20 °C, 20 min at 25 °C or 15 min at 37 °C. Read absorbance A_1, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate approx. 5 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.

To achieve sufficiently precise results, the absorbance differences measured should usually be at least 0.100 absorbance units

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration maltose/sucrose/D-glucose

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

df: dilution factor
RB: Reagent blank

$$df_{basic\ application} = \frac{\text{sample volume} + R1}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df).

$$\mathbf{C_{total\ maltose}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000 \times \mathbf{2})} = \mathbf{0.7063 \times \Delta A}$$

The factor 2 results from the two glucose molecules formed during the hydrolysis of maltose. Take the sample dilution factor into account in the calculation.

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 342.3
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. Calculation of the real maltose concentration after subtraction of sucrose/D-glucose

The result of the E8170 test additionally includes the amounts of free sucrose and free D-glucose that might be present in the sample.
It is calculated using the molecular weight of maltose (342.3 g/mol) and is referred to as *total maltose*.
To determine the real maltose concentration, the sum of sucrose including free D-glucose must be determined using the Enzytec™ *Liquid* Sucrose/D-Glucose assay (E8180).
The result is expressed as *total sucrose* (342.3 g/mol) and subtracted from total maltose for differentiation:

$$C_{maltose}\ [g/L] = C_{total\ maltose\ E8170} - 0.5 \times C_{total\ sucrose\ E8180}$$

Example: Enzytec™ *Liquid* Multi-sugar standard low E8440

Total maltose (E8170)			=	1.225 g/L
Total sucrose (E8180)			=	1.450 g/L
Maltose	=	1.225 g/L - 0.5 × 1.450 g/L	=	**0.500 g/L**

5.1.3. Calculation of the real maltose concentration after differentiation of sucrose and D-glucose

5.1.3.1. Differentiation of sucrose und D-glucose

If differentiation of all three types of sugar is preferred, the free D-glucose must also be determined separately using the Enzytec™ *Liquid* D-Glucose test (E8140) and subtracted from the result of the Enzytec™ *Liquid* Sucrose/D-Glucose test (E8180). The ratio between the molecular weights of the two sugars must be taken into account ($MW_{sucrose}$ 342.3 g/mol, $MW_{glucose}$ 180.16 g/mol).
For further information, please refer to the corresponding package inserts for D-Glucose (E8140) and Sucrose/D-Glucose (E8180).

$$C_{sucrose}\ [g/L] = C_{total\ sucrose\ E8180} - 1.9 \times C_{glucose\ E8140}$$

Example: Enzytec™ *Liquid* Multi-sugar standard low E8440

Total sucrose (E8180)			= 1.450 g/L
D-Glucose (E8140)			= 0.500 g/L
Sucrose	=	1.450 g/L - 1.9 × 0.500 g/L	= **0.500 g/L**

5.1.3.2. Calculation of the maltose concentration

For this calculation, the glucose amount from the calculated real sucrose value (D-glucose + D-fructose) and the measured glucose value adjusted for water content (0.95) is subtracted from the total maltose value.

$$C_{maltose}\ [g/L] = C_{total\ maltose\ E8170} - (C_{sucrose\ E8180} \div 2) - (C_{gluc.\ E8140} \times 0.95)$$

Example: Enzytec™ *Liquid* Multi-sugar standard low E8440

Total maltose (E8170)			= 1.225 g/L
Sucrose (E8180)	=	0.500 g/L ÷ 2 $_{(1x\ D\text{-}glucose)}$	= 0.250 g/L
D-Glucose (E8140)	=	0.500 g/L $_{(D\text{-}glucose)}$ × 0.95 $_{(H2O)}$	= 0.475 g/L
Maltose = 1.225 g/L - (0.500 g/L ÷ 2) - (0.500 g/L × 0.95) = **0.500 g/L**			

5.2. Calculation of solid samples

$$Content_{maltose}\ [g/100\ g] = \frac{C_{maltose}\ [g/L\ sample\ solution]}{weight_{sample}\ in\ g/L\ sample\ solution} \times 100$$

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 the Enzytec™ *Liquid* Multi-sugar standard low (Art. No. E8440) with 0.5 g/L each of maltose, sucrose and D-glucose.
The recovery of maltose control solutions should be 100 ± 5 % and for extracted samples within 100 ± 10 %.

6. Performance data

6.1. Specificity & side activities

The α-glucosidase specifically hydrolyzes α-1,4-glycosidic bonds in maltose, sucrose, maltotriose, maltotetraose and in maltodextrins as well as in other oligo-glucosides such as turanose or maltitol. The β-fructosidase specifically hydrolyzes the β-fructosidic bond of sucrose.
In case of maltotriose, the assay shows a high side activity of 92 %. Maltotetraose reacted at 24 % and maltodextrin at 40 %.
Consequently, it is not possible to distinguish whether maltose, sucrose, D-glucose or other oligosaccharides with α-1,4-glycosidic or β-fructosidic bond are contained in a sample. For differentiation, a parallel test for sucrose and, if necessary, glucose must always be performed (see *calculation of results*). Starch and disaccharides with β-glycosidic bonds such as lactose, lactulose, cellobiose and raffinose as well as disaccharides with α,α-glycosidic bonds such as trehalose and with α-1,6-bonds such as isomaltose and isomaltulose do not react. In the presence of sucrose and D-fructose, a creep reaction may occur if the second incubation time of 5 minutes is exceeded.

6.2. Interferences

Citric acid and ascorbic acid showed no interference at or below 50 g/L. In the case of SO_2, at a concentration of approximately 2 g/L, a slightly increased recovery can be expected.
In the case of D-mannose, strong interference was observed at levels above 7.5 g/L.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 1300 mg/L maltose, whereas the recommended measuring range is between 10 and 1100 mg/L.
The lower limit of detection (LoD) and limit of quantification (LoQ) were determined according to method DIN 32645:2008-11 in buffered aqueous solution for a sample volume of v = 100 µL. This results in an LoD of 5 mg/L and an LoQ of 10 mg/L maltose.
The lowest absorbance difference that the method can distinguish is ΔA = 0.005, resulting in an LoD of 0.5 mg/L for a sample volume of v = 1000 µL. Based on ΔA = 0.010, an LoQ of 1.0 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 on the website 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. Haftungsausschluss

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