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 (36 – 46 °F)

This test was evaluated using selected samples of the following matrices: infant formula, soft drinks, breakfast cereals, corn starch syrup, honey, milk substitute drinks, and beer.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

1. Test principle

The enzyme α-glucosidase (maltase) cleaves maltose and sucrose into two molecules D-glucose and D-glucose and D-fructose, respectively. Moreover, sucrose is 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 adenosine-5'-triphosphate (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^+

Nicotinamide adenine dinucleotide (NAD) is reduced to NADH in this process. The amount of NADH formed during the reaction is equivalent to the amount of sucrose, D-glucose, and half the amount of maltose (= total maltose in g/L) 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, 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 / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

3.1. General

- Sample preparation for manual and automated testing is the same.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with distilled water to a concentration within the measuring range (see performance data).
- Neutralize **strongly** acidic samples to a pH of approx. 7.0 by adding 1 M KOH or NaOH or adding HCl to alkaline samples.
- For turbid test samples: Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbon dioxide (e.g., beer) by stirring in a beaker.
- If necessary, decolorize strongly colored samples with polyvinylpolypyrrolidone (PVPP, e.g., 1 g/100 mL sample). Stir or shake for 1 minute and filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Grind and homogenize solid or semi-solid samples, weigh in suitable sample quantity and extract with water. Filter, centrifuge, or use Carrez clarification if necessary.
- Clarify samples containing proteins (or fat) with Carrez reagents: E.g. weigh or pipette an appropriate quantity into a 100 mL volumetric flask and add approx. 60 mL distilled water. Then add 5 mL Carrez I solution (3.60 g potassium hexacyanoferrate(II)-trihydrate $K_4[Fe(CN)_6]$ x 3 H_2O/100 mL), 5 mL Carrez II solution (7.20 g zinc sulfate $ZnSO_4$ x 7 H_2O/100 mL) and 10 mL 0.1 M NaOH. Mix well after each addition. Fill the measuring flask with distilled water up to the mark, mix and filter (discard first milliliters).
- For samples with a high fat content, weigh e.g. 5 g into a 100 mL volumetric flask, fill halfway with water, and heat in a water bath at 50 – 60 °C (122 – 140 °F) for 20 minutes. After cooling, fill the flask to the mark and place it in the refrigerator for about 20 minutes to separate the fat. Then use a pleated filter to obtain a clear or slightly cloudy sample.

3.2. Determination of maltose in beverages

- Beverages (including beer, Bionade, energy drinks) were used in the test either directly or after dilution with distilled water, depending on the sugar concentration. Degas if neccessary.

3.3. Determination of maltose in bread, pastries and cereal bars

- Weigh approx. 1 g of the homogenized sample to the nearest 1 mg into a 50 mL test tube, add 10 – 20 mL distilled water and vortex vigorously.
- Fill up to 50 ml with distilled water and extract for 30 min at 50 °C with occasional shaking.
- Then allow to cool to room temperature and transfer to 100 mL volumetric flask with distilled water.
- Successively add 1 mL concentrated Carrez I solution (150 g/L potassium hexacyanoferrate) and 1 mL concentrated Carrez II solution (300 g/L zinc sulfate) and mix after each addition.
- Finally, fill up to the final volume with distilled water, shake and filter through a pleated filter.

3.4. Determination of maltose in meat

The following processing recommendation refers to meatballs as an example of meat and sausage products.

3.4.1. Preparation according to §64

- Weigh approx. 10 g of the homogenized sample to the nearest 1 mg into 50 mL test tube, add 20 mL distilled water and vortex vigorously.

- Fill up to 50 mL with distilled water and heat for 15 min at 70 °C in a water bath. Then add 1 drop of concentrated sulphuric acid (98 %) and transfer to a 100 mL volumetric flask.
- Allow the sample to cool to room temperature and fill up to the final volume with distilled water (fat above the calibration mark), mix carefully and filter through a pleated filter.

3.4.2. Adapted preparation for samples containing starch

- Weigh 1 – 1.5 g of the homogenized sample to the nearest 1 mg into an Erlenmeyer flask, add 20 mL of DMSO, stir briefly and add 5 mL of 25 % HCl.
- Seal with a stopper or parafilm and stir for 60 min at 60 °C in a water bath or on a hotplate.
- Cool rapidly to room temperature in an ice bath and transfer to a 100 mL volumetric flask with 0.1 M citrate buffer pH 4.0.
- Add 5 mL 8 M NaOH and, after cooling again to room temperature, fill up to the final volume with the citrate buffer and filter through a pleated filter.

4. Manual test procedure

Wavelength:	340 nm
Temperature (measurement):	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
Measuring range:	10 – 1100 mg/L (for 100 µL)

	Reagent blank	**Sample / control**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate approx. **30 minutes at 20 °C (68 °F), 20 minutes at 25 °C (77 °F) or 15 minutes at 37 °C (99 °F). Read absorbance A_1**, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate for **5 minutes at 20 – 37 °C (68 – 99 °F)** and **read absorbance A_2**.		

4.1. Important notes for assay procedure

- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Specified incubation times were validated at 37 °C (99 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- Always wait for the reaction to end or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped after the recommended incubation time, continue measuring at 5-minute intervals, for example, until a constant absorbance value is reached.
- If a creep reaction occurs, the reaction will not have finished after stated incubation times and will typically show a constant increase of ΔA. Calculate the analyte-specific ΔA value by plotting the absorbance values against time and performing a linear regression to determine the rate of increase in ΔA per minute related to the creep reaction. Then, extrapolate the absorbance to the time at which reagent 2 is added.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration maltose/sucrose/D-glucose

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume} + R1}{\text{test volume}} = 0.808$$

The specified df value of **0.808 applies to a base application of 100 µL**. An increase in sample volume is possible (max. 1000 µL; refer to validation report). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df)** accordingly.

Increasing the sample volume may influence test performance. This must generally be checked depending on the matrix. **The reagent blank value must be adjusted to the changed sample volume.**

The concentration of total maltose is calculated using Lambert-Beer's law:

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

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.

If the sample solution was diluted before measurement, this result has to be multiplied with the sample **pre-dilution factor F.**

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight maltose [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 Enzytec™ *Liquid* Maltose/Sucrose/D-Glucose E8170 test result also includes the amounts of free sucrose and free D-glucose that may be present in the sample.

This is calculated using the molecular weight of maltose (342.3 g/mol) and is referred to as total maltose.

To determine the actual maltose concentration, the sum of sucrose including free D-glucose must be determined using the Enzytec™ *Liquid* Sucrose/D-Glucose assay E8180.

This is expressed as total sucrose (342.3 g/mol), which is then subtracted from total maltose to make the distinction:

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

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 the three types of sugar is required, the concentration of free D-glucose must be determined separately using the test Enzytec™ Liquid D-Glucose E8140, and this value must then be subtracted from the result of the test Enzytec™ Liquid Sucrose/ D-Glucose E8180. The ratio of the molecular weights of the two sugars ($MW_{sucrose}$ = 342.3 g/mol and $MW_{glucose}$ = 180.16 g/mol) must be taken into account.

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

For further information, please refer to the corresponding package inserts for D-Glucose (E8140) and Sucrose/D-Glucose (E8180).

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

In this calculation, the amount of glucose is obtained by subtracting the calculated real sucrose value (D-glucose + D-fructose) and the measured glucose value, adjusted for water content (0.95), 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-glucose)	= 0.250 g/L
D-Glucose (E8140)	=	0.500 g/L (D-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**

Important note: The precision of the maltose and sucrose determination is impaired if the ratio of D-glucose to maltose and sucrose is higher than e.g. 10:1. In this case, as much as possible of the D-glucose should be removed using the Enzytec™ Glucose Remover E3400 kit.

5.2. Calculation of solid samples

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weight:

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

5.3. Controls & acceptance criteria

Control or reference samples should be included in each run for quality control purposes. Therefore, we recommend Enzytec™ *Liquid* Multi-Sugar Standard *low* (Art. No. E8440 with 0.5 g/L each of maltose, sucrose and D-glucose).

The recovery of this multi-standard low and other aqueous control solutions should be 100 ± 5 %, and for extracted samples within 100 ± 10 %.

As a certified (standard) reference material, we recommend:

- NIST SRM3233 breakfast cereals
- NIST SRM1869 infant/adult/nutritional formula 2 (milk/whey/soy-based)

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 case of sulfite (SO_2), at a concentration of approximately 2 g/L, a slightly increased recovery can be expected.

In case of D-mannose, a 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 (sample volume of 100 µL) with a recommended measuring range of 10 – 1100 mg/L.

The limit of detection (LoD) was determined according to the DIN 32645:2008-11 method in a buffered aqueous solution. For a sample volume of 100 µL, the calculated LoD is 5.0 mg/L.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 10.0 mg/L for a sample volume of 100 µL.

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. 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* Maltose/Sucrose/D-Glucose Validation Report
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Maltose/Sucrose/D-Glucose Excel template for results
- Enzytec™ *Liquid* Maltose/Sucrose/D-Glucose Technical information
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

Upon request, we offer the following services, among others:

- Customized troubleshooting
- Workflow analysis
- Data & results analysis
- Customer workshops & webinars
- Automation: application support & technical service

10. Disclaimer

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