UV assay for the determination of maltose, sucrose and D-glucose in foodstuffs and other sample materials
Test combination for 25 determinations each

For *in vitro* use only
Store between 2 – 8 °C (36 – 46 °F)

This test kit contains all the reagents required for the separate determination of maltose, sucrose and D-glucose in up to three cuvettes (see test principle).

The test was evaluated using selected samples of the following matrices: cereals, baby food, baked goods, muesli bars, soft drinks, beer and meat.

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

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

## 1. Test principle

The result of the first (1) cuvette (maltose sample) is calculated using the molecular weight of the maltose (342.3 g/mol) and is referred to as total maltose (maltose sample). This contains the amounts of free sucrose and free D-glucose that could be present in the sample.

(1) The enzyme α-glucosidase (maltase) splits maltose and sucrose into two molecules of D-glucose or D-glucose and D-fructose, respectively:

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

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

To determine the apparent or real maltose concentration, the sum of sucrose including free D-glucose must be determined in a second (2) cuvette (sucrose sample), which is referred to as total sucrose. The result is expressed in terms of the molecular weight of sucrose (342.3 g/mol) and then subtracted from the total maltose to achieve differentiation.

(2) Sucrose is hydrolyzed to D-glucose and D-fructose by the enzyme β-fructosidase (invertase):

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

To differentiate between all three types of sugar, free D-glucose (180.16 g/mol) must also be determined in a third (3) cuvette (glucose sample) and subtracted from the result for total sucrose. The ratio between the molecular weights must be taken into account.

(3) In all cuvettes, D-glucose is phosphorylated with adenosine triphosphate (ATP) in the presence of the enzyme hexokinase (HK) to form D-glucose-6-phosphate (G-6-P), resulting in adenosine diphosphate (ADP):

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

In the presence of glucose-6-phosphate dehydrogenase (G6P-DH), D-glucose-6-phosphate is oxidized to D-gluconate-6-phosphate, and nicotinamide adenine dinucleotide (NAD) is simultaneously reduced to NADH:

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

The amount of NADH formed during the reaction is equivalent to the amount of sucrose, the amount of D-glucose, and half the amount of maltose, 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 manual processing 25 determinations each of maltose, sucrose and glucose. The number of determinations for automated processing is increased by a multiple; however it depends on the device

- Reagent 1: 1 x 50 mL mit Puffer, NAD, α-glucosidase
- Reagent 2: 1 x 50 mL mit Puffer, NAD, β-fructosidase
- Reagent 3: 1 x 50 mL mit Puffer, NAD
- Reagent 4: 1 x 37.5 mL mit Puffer, hexokinase, 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 identical.
- Samples solutions should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or dilute sufficiently to yield a concentration within the stated measuring range (refer to performance data).
- 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 by, for example, stirring them in a beaker or applying a brief ultrasonic pulse (10 s).
- If necessary, decolorize **strongly** colored and highly concentrated samples with polyvinyl-polypyrrolidone (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.
- Crush and homogenize solid and semi-solid samples. Weigh a sufficient quantity of sample in a volumetric flask (considering the measuring range), extract with water; fill up to the mark and filter if necessary (by using fluted paper or syringe filters) or centrifuge in reaction tubes. Use Carrez clarification if necessary.
- For samples with a high fat content, weigh sufficient quantity (considering the measuring range) into a volumetric flask and extract with hot water. Cool to allow the fat to separate, fill to the mark, place the volumetric flask in an ice bath for 15 minutes and filter.
- Clarify samples containing proteins or fat alternatively with Carrez reagents: Weigh an appropriate sample quantity accurately into a 100 mL volumetric flask and add approx. 60 mL distilled water. In case of liquid samples, pipette the sample into a 100 mL volumetric flask or beaker pre-filled with 60 mL distilled water. Add 5 mL Carrez I solution (3.60 g potassium hexacyano-ferrate(II)-trihydrate $K_4[Fe(CN)_6]$ x 3 $H_2O$/100 mL) and 5 mL Carrez II solution (7.20 g zinc sulfate $ZnSO_4$ x 7 $H_2O$/100 mL). Mix well after each addition. Adjust the pH with 0.1 M NaOH to a value between 7.5 and 8.5. Transfer into a 100 mL volumetric flask, fill up to the mark, mix and filter using fluted paper filters or syringe filters.
  Alternatively, clarify using perchloric acid.
- In case of higher sample volumes (up to 1000 µL), check the pH value of the test solution and neutralize in case of any doubt.

### *3.2. Determination of maltose in beverages*

- Use beverages (including beer, Bionade, and energy drinks) in the test either undiluted or after diluting them with distilled water, depending on their sugar concentration.
- Degas carbonated samples beforehand.

### *3.3. Determination of maltose in bread, rolls, 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 minutes at 50 °C (122 °F) with occasional shaking.
- Then allow to cool to room temperature and transfer to 100 mL volumetric flask with distilled water.
- Successively add 1 mL Carrez I solution (150 g/L potassium hexacyanoferrate) and 1 mL Carrez II solution (300 g/L zinc sulphate) 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 German §64 LFGB*

- Weigh approx. 10 g of the homogenized sample to the nearest 1 mg into 50 mL test tube, add 20 mL dist. water and vortex vigorously.
- Fill up to 50 mL with distilled water and heat for 15 minutes at 70 °C (158 °F) in a water bath. Then add 1 drop of concentrated sulphuric acid (98 %) and transfer to a 100 mL volumetric flask with distilled water.
- Allow the sample to cool to room temperature and fill up to the final volume with dist. 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 minutes at 60 °C (140 °F) 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 – 1000 mg/L (for 100 µL) |

| | Maltose | | Sucrose | | D-Glucose | |
|---|---|---|---|---|---|---|
| | **RB** | **Sample** | **RB** | **Sample** | **RB** | **Sample** |
| **Reagent 1** | 2000 µL | 2000 µL | - | - | - | - |
| **Reagent 2** | - | - | 2000 µL | 2000 µL | - | - |
| **Reagent 3** | - | - | - | - | 2000 µL | 2000 µL |
| **Sample** | - | 100 µL | - | 100 µL | - | 100 µL |
| **dist. $H_2O$** | 100 µL | - | 100 µL | - | 100 µL | - |
| Mix, incubate for **30 minutes at 20 – 25 °C (68 – 77 °F)** or **20 minutes at 37 °C (99 °F)**. **Read** absorbance **$A_1$**, then add: | | | | | | |
| **Reagent 4** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| Mix, incubate for **20 minutes at 20 – 25 °C (68 – 77 °F)** or **15 minutes at 37 °C (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 and determined at 37 °C (98 °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 several times 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 4 (HK/G6P-DH) is added.
- To obtain a sufficiently accurate result, the measured absorbance difference should be at least 0.020.
- 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 concentrations in sample solutions*

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. **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 respective concentrations are calculated using Lambert-Beer's law:

$$C\ [g/L] = \frac{V \times MW}{(\varepsilon \times d \times v \times 1000)} \times \Delta A \times F$$

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] | = 2.6 |
| MW: | Molecular weight of the substance to be determined | |
| | Maltose [g/mol] | = 342.3 |
| | Sucrose [g/mol] | = 342.3 |
| | D-glucose [g/mol] | = 180.16 |
| d: | Optical path [cm] | = 1.0 |
| v: | Sample volume [mL] | = 0.1 |
| ε: | Extinction coefficient NADH [L/mmol x cm] | = 6.3 (at 340 nm) |

#### *5.1.1. Calculation of* apparent *maltose,* apparent *sucrose and D-glucose concentrations*

The following calculation formulas result from Lambert-Beer's law:

**(I) Cuvette 1 – Total maltose:**

$$C_{\text{total maltose}}\ [g/L] = \frac{(2.6 \times 342.3 \times \Delta A)}{(6.3 \times 1 \times 0.1 \times 1000 \times 2)} = \mathbf{0.7063 \times \Delta A} \times F$$

The factor 2 only applies to the determination of maltose and results from the two glucose molecules that are formed during hydrolysis.

**(II) Cuvette 2 – Total sucrose:**

$$C_{\text{total sucrose}}\ [g/L] = \frac{(2.6 \times 342.3 \times \Delta A)}{(6.3 \times 1 \times 0.1 \times 1000)} = \mathbf{1.4127 \times \Delta A} \times F$$

**(III) Cuvette 3 – (free) D-glucose:**

$$C_{\text{D-glucose}}\ [g/L] = \frac{(2.6 \times 180.16 \times \Delta A)}{(6.3 \times 1 \times 0.1 \times 1000)} = \mathbf{0.7435 \times \Delta A} \times F$$

If the sample solutions were diluted before measurement, the results have to be multiplied with the **sample pre-dilution factor F**.

#### *5.1.2. Calculation of the actual concentration of maltose*

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

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

| | | | | |
|---|---|---|---|---|
| total maltose | | | = | 1.225 g/L |
| total sucrose | | | = | 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 actual concentration of sucrose*

$$C_{\text{sucrose}}\ [g/L] = C_{\text{total sucrose}} - (1.9 \times C_{\text{D-glucose}})$$

The factor 1.9 takes into account the water content of the D-glucose units.

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

| | | | | |
|---|---|---|---|---|
| apparent sucrose | | | = | 1.450 g/L |
| D-glucose | | | = | 0.500 g/L |
| real sucrose | = | 1.450 g/L - 1.9 × 0.500 g/L | = | **0.500 g/L** |

#### *5.1.4. Calculation of the maltose concentration from the individual sugar concentrations*

$$C_{\text{real maltose}}\ [g/L] = C_{\text{apparent maltose}} - (0.5 \times C_{\text{real sucrose}}) - (0.95 \times C_{\text{D-glucose}})$$

The factor 0.95 takes into account the water content of the D-glucose.

### *5.2. Calculation for solid samples*

When analyzing solid and semi-solid samples that are weighed in for sample preparation, the analysis result is based on the sample weight:

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

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

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

**Example:**

$C_{\text{maltose}}$ = 0.274 g/L Weigh in = 5.02 g/100 mL ≙ 50.2 g/L

$$\text{Content}_{\text{maltose}} = \frac{0.274\ g/L}{50.2\ g/L} \times 100 = 0.546\ g/100\ g \text{ (or \%)}$$

### *5.3. Controls & acceptance criteria*

Control or reference samples should be included in every run for quality control purposes. For this purpose, we recommend the use of Enzytec™ *Liquid* Multi-sugar standard *low* (E8440) with each 0.5 g/L maltose, sucrose and D-glucose (see sample calculations in chapter 5.1. Calculation of sample solutions).

The recovery of this multi standard and other *aqueous* control solutions should be 100 ± 5 %.

## 6. Performance data

### *6.1. Specificity & side activities*

The α-glucosidase 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 hydrolyzes the β-fructosidic bond of sucrose.

The test shows a high secondary activity of up to 92 % against maltotriosis. Maltotetraose, -pentaose and -hexaose reacted to a maximum of 4 %. (Malto-)dextrins reacted to a maximum of 11 %.

Starch and disaccharides with β-glycosidic bonds such as lactose, lactulose, cellobiose and raffinose as well as disaccharides with α,α-glycosidic bonds (e.g. trehalose) and with α-1,6 bonds (e.g. isomaltose and isomaltulose) do not react.

### *6.2. Interferences*

Citric acid and L-ascorbic acid showed no interference at or below 50 g/L. D- and L-lactic acid showed no interference at or below 25 g/L, while D- and L-malic acid showed no interference at or below 5 g/L. In the case of $SO_2$, a slightly increased recovery is to be expected at a concentration of approx. 2 g/L.

Fructose, lactose, lactulose, D-mannose, trehalose and raffinose do not interfere up to at least 5 g/L.

### *6.3. Linearity, measuring range & sensitivity*

The linearity is given up to 1000 mg/L total maltose, whereby the recommended measuring range is between 10 and 1000 mg/L.

The lower limit of detection (LoD) and the limit of quantification (LoQ) were determined according to the method DIN 32645:2008-11 in buffered aqueous solution for a sample volume of v = 100 µL. This results in an LoD of 2.3 mg/L and an LoQ of 3.9 mg/L maltose.

## 7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid Combi Maltose/Sucrose/D-Glucose* E8175 Excel template for results
- Enzytec™ *Liquid Combi Maltose/Sucrose/D-Glucose* E8175 Technical information
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form at 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

On request, we offer the following services:

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

## 10. Disclaimer

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