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

**This test contains all reagents for the differentiated determination of maltose, sucrose and D-glucose. The concentrations of the three individual sugars are thus determined in up to three separate cuvettes (see test principle).**

This test was examined with the following matrices: cereals, baby food, baked goods, muesli bars, soft drinks, beer and meat. Detailed results and further information on the validation data can be found in the validation report.

Other foods or sample materials can be tested and must be validated by the user.

## 1. Test principle

The result of the *first* cuvette is calculated using the molecular weight of the maltose (342.3 g/mol) and is referred to as apparent 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 actual maltose concentration, the sum of sucrose including free D-glucose must be determined in a *second* cuvette, which is referred to as apparent sucrose (sucrose sample). The result is expressed with the molecular weight of the sucrose (342.3 g/mol) and subtracted from the apparent maltose for 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, the free D-glucose (180.16 g/mol) must also be determined separately in a *third* cuvette and subtracted from the result for apparent sucrose.

The ratio between the molecular weights of the two sugars 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 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 each case, nicotinamide adenine dinucleotide (NAD) is reduced to NADH. The amount of NADH formed is proportional to the amount of D-glucose present and is measured at 340 nm in each case.

## 2. Reagents

### *2.1. Content & composition*

The test is suitable for manual and automated processing. The reagents are sufficient for manual processing for 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: Maltose | 1 x 50 mL | buffer, NAD, α-glucosidase |
| • Reagent 2: Sucrose | 1 x 50 mL | buffer, NAD, β-fructosidase |
| • Reagent 3: D-Glucose | 1 x 50 mL | buffer, NAD |
| • Reagent 4: | 1 x 37.5 mL | buffer, hexokinase, 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 printed expiration date (see label) even after opening at 2 - 8 °C, if handled properly. Do not freeze reagents.

### *2.4. Safety & disposal*

The product/test is only suitable for use within the scope of its intended purpose. The instructions for use must be strictly followed.

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

Safety instructions for the components contained can be found in the respective safety data sheets (SDS). After use, the reagents can be disposed of with the laboratory waste and the packaging material can be recycled.

## 3. Sample preparation

### *3.1. General*

- Sample preparation for manual and automated testing is identical.
- Bring samples 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 cloudy solutions using a pleated filter and, if necessary, a syringe filter.
- Degas samples containing carbon dioxide, e.g. by filtration or centrifugation.
- Strongly colored and highly concentrated samples should be decolorized with polyvinylpolypyrrolidone (PVPP).
- Clarify protein-containing samples with Carrez reagents or alternatively with perchloric acid.
- Weigh samples with a high fat content into a volumetric flask and extract with hot water; allow the sample solution to cool for fat separation (e.g. 15 min in an ice bath); fill the volumetric flask up to the mark with water and filter before testing.
- Sufficiently homogenize and crush solid and semi-solid samples; extract with water or dissolve in dist. water and filter if necessary.

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

- Beverages (including beer, Bionade, energy drinks) were used in the test either directly or after dilution with dist. water, depending on the sugar concentration (degas carbonated drinks by stirring in a beaker).

### *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 dist. 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 dist. 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 dist. 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.

**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 dist. water and vortex vigorously.
- Fill up to 50 mL with dist. 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 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.

**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.

### *3.5. Further instructions on sample preparation*

Use separate tips for each sample extract (and the control solutions) to avoid cross-contamination; rinse the tip with the extract before pipetting.

## 4. Assays performance

| | |
|---|---|
| Wavelength: | 340 nm |
| Temperature: | 20 - 37 °C (during the measurement) |
| Photometer alignment: | against air (without cuvette) |
| Measuring range: | 10 - 1000 mg/L (apparent maltose) |

| | 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 min at 20 - 25 °C or 20 min at 37 °C. Measure absorbance $A_1$, then add: | | | | | | |
| **Reagent 4** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| Mix, incubate for 20 min at 20 - 25°C or 15 min at 37 °C and measure absorbance $A_2$. | | | | | | |

- The reagent blank (RB) must be determined **once** for each parameter in each run and subtracted from each sample result for the corresponding parameter.
- Reagent blank and sample must be measured **in the same run** and under the same conditions.
- To increase sensitivity, the sample volume can be increased by up to 1000 µL (see validation report) with **unchanged** reagent volumes.
- The volume of the reagent blank must be adjusted to the changed sample volume.
- Increasing the sample volume may affect the test system. In general, this must be checked depending on the matrix.

### *4.1. Further advice on carrying out the test*

- The specified incubation times may vary depending on the prevailing laboratory conditions and the pipetting accuracy. It is therefore recommended to wait for the end of the reaction during the first runs and to adjust the times if necessary.
- If the reaction has not stopped after the specified incubation time, the absorbances should be measured at 2 min intervals until a constant increase in absorbance per 2 min is achieved. If constant absorbance increases were observed, the absorbances $A_2$ are extrapolated to the time of addition of reagent 4 (HK/G6P-DH).
- To obtain a sufficiently precise result, the measured absorbance differences should be at least 0.02 absorbance units.
- The use of a multistepper pipette is recommended for the addition of reagents. Use a separate tip for each component and rinse the tip with the respective reagent before pipetting.
- The use of stirring spatulas for each individual cuvette is recommended for mixing. Only remove this from the cuvette immediately before the absorbance measurements.
- If the measured absorbance difference is too small (e.g. $\Delta A < 0.02$), increase the sample volume (v) to a maximum of 1000 µL or prepare the sample solution again (higher weight or less dilution).
  In this case, the volume of water in the reagent blank must be adjusted accordingly so that the same test volume is present in the sample and blank preparations.
  The changed sample volume must be used accordingly in the calculation formulae.

## 5. Calculation of results

### *5.1. Calculation of sample solutions*

The extinction difference ΔA must be calculated for each sample:

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

df: dilution factor
RB: reagent blank

$$df_{100\mu L} = \frac{\text{sample volume + R1}}{\text{test volume}} = \mathbf{0.808}$$

The stated df value of 0.808 applies to a basic application of 100 µL. **Increasing the sample volume requires conversion of the reagent dilution factor (df).**

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

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

| | | |
|---|---|---|
| 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) |

If the sample solution was diluted before the measurement, the result must be multiplied by the **pre-dilution factor F**.

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

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

**Cuvette 1 – apparent maltose:**

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

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

**Cuvette 2 – apparent sucrose:**

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

**Cuvette 3 – (free) D-glucose:**

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

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

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

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

| | | | | |
|---|---|---|---|---|
| apparent maltose | | | = | 1.225 g/L |
| apparent sucrose | | | = | 1.450 g/L |
| real 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*

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

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:

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

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

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

**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 maltose 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* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Technical information
- 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, the individual test procedure and the laboratory environment. Detection and quantification limits depend on the respective sample matrix and the extraction method. For detailed results and further information, please refer to the current validation report.

For the present enzymatic test, only stated, exemplary matrices could be validated due to the large number of foodstuffs and other sample materials.

When analyzing a non-validated matrix, it is recommended to verify the results obtained by means of spike experiments. If necessary, a validation of the sample matrix of interest will need to be performed.

## 9. Services & technical support

On request, we offer the following services:

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

## 10. Disclaimer

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