Sucrose/D-Glucose, colorimetric method

Catalogue number: AK00241, 250 tests of each analyte

Application
This rapid and simple specific enzymatic method is used for the simultaneous determination of sucrose and D-glucose in foodstuffs, pharmaceuticals, cosmetics and biological samples.

Introduction
Sucrose and D-glucose occur widely in plant organisms. In foods, they occur mainly in honey, wine and beer, and a range of solid foodstuffs such as bread and pastries, chocolate and candies. In the wine industry, the addition of sucrose is only allowed in few situations, such as champagne production.

Principles
The D-glucose concentration is determined before and after hydrolysis of sucrose by β-fructosidase. The sucrose content is calculated from the difference in D-glucose concentrations before and after hydrolysis by β-fructosidase. Free D-glucose in the sample extract is determined by conversion to a red coloured quinoneimine dye compound through the combined action of glucose oxidase and peroxidase.

Specificity
Using high purity of glucose oxidase, peroxidase and β-fructosidase, this colorimetric method is specific for sucrose and D-glucose measurement in plant and food extracts. The colour formed through the reaction is stable at room temperature for at least 2 hours after development.

Linearity and precision
Linearity of the determination exists from 10 to 100 μg D-glucose or sucrose per assay (see Figure 1). Standard errors below 5% are reached routinely.

Kit composition
Solution 1. Buffer (20 mL, pH 4.6). Stable for 2 years at 4 °C.
Dilute the content of bottle 1 to 100 mL with distilled water before use. Stable for > 1 year at 4°C.

Suspension 2. β-Fructosidase (5 mL). Stable for 2 years at 4 °C. Swirl bottle before use.
Add 0.02 ml of Suspension 2 plus 0.180 ml of Solution 1, per assay, to a test tube and homogenise (Solution 1+2). This solution should be prepared for each assay day.

Solution 3. GOD-POD reagent buffer (2 x 22.5 mL, pH 7.4), p-hydroxybenzoic acid and sodium azide (0.64% w/v) as a preservative. Stable for 3 years at 4 °C.
Dilute the contents of one bottle to 0.75 L with distilled water and use immediately.

Mixture 4. GOD-POD reagent enzymes. Freeze-dried powder of glucose oxidase (GOD), peroxidase (POD) and 4-aminoantipyrine. Stable for 5 years at -20 °C.
Dissolve the contents of one bottle 4 in approx. 20 mL of solution 3 and quantitatively transfer this to the bottle containing the remainder of solution 3. Cover this bottle with aluminum foil to protect the enclosed reagent from light. Stable for 3 months at 2-5 °C or 12 months at -20 °C.

Solution 5. D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid. Stable for 5 years at room temperature.

Safety
The general safety measures that apply to all chemical substances should be followed. For more information regarding the safe usage of this kit please refer to MSDS available at www.nzytech.com.
**Procedure (endpoint analysis)**

Wavelength: 510 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~50 °C

Final volume: 3.3 mL

Sample solution: 10-100 μg of total sucrose and D-glucose per cuvette

Read against reagent blank

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<table>
<thead>
<tr>
<th>Pipette into cuvettes (mL)</th>
<th>Blank</th>
<th>D-Glucose Standard</th>
<th>For each sample</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>D-Glucose assay (A)</td>
</tr>
<tr>
<td>Solution 1+2*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Solution 1</td>
<td>-</td>
<td>-</td>
<td>0.20</td>
</tr>
<tr>
<td>H₂O</td>
<td>0.30</td>
<td>0.20</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix. Incubate for 20 min at 50 °C. Then add:

GODPOD reagent (3+4)

Mix, incubate at 50 °C for 20 min and read absorbances at 510 nm against the reagent blank to obtain ΔA_D-glucose standard, ΔA_sample A and ΔA_sample B

Mixtures can be obtained with a plastic spatula or by gentle inversion after sealing with a cuvette cap or Parafilm®.

*Pipette both Solution 1+2 and sample into the bottom of the cuvette and mix by gentle swirling

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**Calculation**

The concentration of D-glucose and sucrose (g/L) are calculated as follows:

\[
[D-\text{Glucose}] = \frac{\Delta A_{\text{Sample} \ (A)}}{\Delta A_{D-\text{Glucose Standard}}} \quad [\text{g/L}]
\]

\[
[Sucrose] = \frac{\Delta A_{\text{Sample} \ (B)} \times \frac{342}{180}}{\Delta A_{D-\text{Glucose Standard}}} \quad [\text{g/L}]
\]

Sucrose calculation takes in account the conversion of μg of D-Glucose (as measured) to μg of sucrose

If the sample has been diluted or a different sample volume was used during the reaction, the result must be multiplied by the corresponding dilution/concentration factor.

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**Interferences**

If the concentration of D-glucose in the sample is much larger than D-fructose (e.g. 10x higher), then the precision of sucrose and D-fructose is compromised. In this situation, the content of D-glucose should be reduced using glucose oxidase/catalase reagent in the presence of oxygen (see Sample Preparation).

An internal standard should be included during sample analysis if the presence of interfering substances is suspected. A quantitative recovery of this standard should be expected.

With each new batch of GODPOD Reagent, the time of maximum colour formation with 100 μg of D-Glucose standard should be checked. This is approximately 15 min. (See Figure 2)

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**Figure 1.** Linearity of the assay. Standard curves relating D-Glucose and Sucrose concentration (μg/test) to absorbance at 510 nm (25 °C)
Determination of sucrose: if the estimated sucrose concentration of the honey lies between 5 and 10%, a dilution 1:3 of the 1% (W/V) honey solution and sample volume of 0.1 mL are satisfactory. If the estimated sucrose concentration of the honey lies below 5%, D-glucose should be removed (see below), otherwise the precision of the sucrose determination will be compromised.

Determination of sucrose and D-glucose in jam

Homogenise about 10 g of jam in a mixer. Pour approx. 0.5 g of the sample accurately weighed, into a 100 mL volumetric flask. Dissolve initially with only a small volume of distilled water (~50 mL) and then dilute to the mark, mix and filter. Discard the first 5 mL of filtrate. Typically, no dilution will be required and a sample volume of 0.1 is satisfactory.

Special sample preparation for the determination of sucrose and D-fructose in the presence of excess of D-glucose

Pipette to a 25 mL volumetric flask: 5 mL of buffer (300 mM sodium phosphate plus 5 mM MgCl₂, pH 7.6), 5 mL of sample solution and 0.2 mL of enzyme solution (glucose oxidase 600 U/mL plus catalase 15000 U/mL). Incubate at ~25 °C and pass a current air (O₂) through the mixture for 1 h. After the reaction, inactivate the enzymes by incubation the flask in a boiling water bath for 10 min. After cooling, dilute the content to the mark with distilled water. Mix and filter. Use 0.5 mL of the clear solution for the determination of sucrose. Determine residual D-glucose as habitual.

References


Examples of sample preparation

Determination of sucrose and D-glucose in fruit juices

Filter turbid juices or clarify using Carrez reagents. Dilute to give a sugar concentration of approximately 0.1-1.0 g/L. Strong coloured samples should be treated with PVPP (see above). Slightly coloured samples can be assayed directly. Typically, for orange and apple juice, a dilution of 1:100 and a sample volume of 0.1 mL are satisfactory.

Determination of sucrose and D-glucose in honey

After stirring the honey sample thoroughly with a spatula, transfer approx. 10 g of the viscous or crystalline material to a beaker and heat for 15 min at approx. 60 °C and stir (there is no need to stir liquid honey). After cooling, prepare 1% (w/v) honey solution: pour approx. 1 g of the liquid sample accurately weighed, into a 100 mL volumetric flask. Dissolve initially with only a small volume of distilled water and then dilute to the mark and mix.
### Certificate of Analysis

<table>
<thead>
<tr>
<th>Test</th>
<th>Criteria</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Performance</td>
<td>Reaction completed within time stated</td>
<td>Meets specification</td>
</tr>
<tr>
<td></td>
<td>Target value for recommended standard material +/− 10%</td>
<td>Meets specification</td>
</tr>
<tr>
<td>Blank reaction absorbance</td>
<td>+/− 10% of the blank value</td>
<td>Meets specification</td>
</tr>
</tbody>
</table>

Approved by: José Prates  
Senior Manager, Quality Systems

Please enquire info@nzytech.com to obtain any additional information about this kit, including additional specific applications.