Application
This rapid and simple specific enzymatic method is used for the determination of D-glucose in foodstuffs such as baking agents, diet beer and dietetic food, as well as in pharmaceuticals, cosmetics and biological samples. The analysis of D-glucose in foodstuffs is normally performed in conjunction with D-fructose, maltose and sucrose (for further information on literature or analytical kits, please contact Nzytech).

Introduction
D-Glucose occurs widely in plants and animals. It is an essential component of carbohydrate metabolism and occurs frequently in the free form along with D-fructose and sucrose. However, the more important forms are those of di- (lactose, maltose, sucrose), tri-, oligo- and polysaccharides (dextrins, starch, cellulose). It is present in significant quantities in honey, wine and beer, and a range of solid foodstuffs such as bread and pastries, chocolate and candies. Measurement of D-glucose is extremely important in biochemistry and clinical analysis, as well as in food analysis; it is mostly determined along with other carbohydrates.

Principle
D-glucose + ATP $\rightarrow$ Glucose-6-P (G6P) + ADP
G6P + NADP$^+$ $\rightarrow$ D-Gluconate-6-P + NADPH + H$^+$

The amount of NADPH formed through the combined action of hexokinase (HK) and glucose-6-P desidrogenase (G6PDH), measured at 340 nm, is stoichiometric with the amount of D-glucose in sample volume.

Specificity
This method is specific for D-glucose since the hexokinase used has only glucokinasic activity.

Sensitivity and detection limit
The sensitivity of the assay is based on 0.010 AU and a sample volume of 2.00 ml. This corresponds to a D-glucose concentration of 0.33 mg/l sample solution when measured at 340 nm. The detection limit of 0.66 mg/l is derived from the absorbance difference of 0.020 (340 nm) and a maximum sample volume of 2.00 ml.

Linearity and precision
Linearity of the determination exists from 4 to 80 μg D-glucose per assay (v = 2.00 ml). In a double assay using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of 2.00 ml, this corresponds to a D-glucose concentration of approx. 0.17 to 0.33 mg/L. The coefficient of variation is approx. 1 to 2 %.

Kit composition
Solution 1. Imidazole buffer (25 mL, 2 M, pH 7.6) plus MgCl$_2$ (0.5 M), MgCl$_2$ (100 mM) and sodium azide (0.02 % w/v) as a preservative. Stable for 2 years at 4 °C.

Solution 2. NADP$^+$ (150 mg) plus ATP (440 mg). Stable for 5 years at -20 °C.

Dissolve in 12 mL of distilled water, divide into appropriately sized aliquots and store in PP tubes at -20 °C between use (stable for 2 years) and keep cool during use.

Suspension 3. Hexokinase (EC 2.7.1.1; 425 U/mL) and glucose-6-P dehydrogenase (EC 1.1.1.49; 212 U/mL) in 3.2 M ammonium sulphate (2.25 ml). Stable for 2 years at 4 °C.

Solution 4. D-Glucose standard solution (6 mL, 0.40 mg/mL) in 0.02% benzoic acid. Stable for 2 years at room temperature.

This standard solution can be used when there is some doubt about the method accuracy ($\varepsilon_{\text{NADH,340 nm}} = 6300 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$).

Safety
Reagents that are used in the determination of D-glucose are not hazardous materials (see Hazardous Substances Regulations). However, the concentrated buffer contains sodium azide as a preservative. The general safety measures that apply to all chemical substances should be followed.

Procedure (endpoint analysis)
Read absorbances against air, or water, at 340 nm

<table>
<thead>
<tr>
<th>Pipette into cuvettes (mL)</th>
<th>Blank</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (at ~25 °C)</td>
<td>2.10</td>
<td>2.00</td>
</tr>
<tr>
<td>Sample (can vary 0.10-2.00 ml)</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>Solution 1 (imidazole buffer)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Solution 2 (NADP$^+$+ATP)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix, measure the absorbances of the above solutions (A1) after approx. 3 min and start the reaction by addition of

| Suspension 3 (HK+G6PDH) | 0.02 | 0.02 |

Mix, measure the absorbances of the above solutions (A2) at the end of the reaction (approx. 5 min)*

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

* if necessary, continue to read the absorbances at 2 min intervals until the reaction ends.

Calculation

D-Glucose, UV method

Catalogue number: AK00031, 110 tests per kit
Determine the absorbance difference for both blank and sample (A2-A1). Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{D\text{-glucose}}$. The concentration of D-glucose (g/L), based on the $\varepsilon$ of NADH at 340 nm (6300 L×mol$^{-1}$×cm$^{-1}$), is calculated as follows: $0.6634 \times \Delta A_{D\text{-glucose}}$. If the sample has been diluted during preparation, the result must be multiplied by the dilution factor.

Interferences
If the conversion of D-glucose completes within the time specified in the assay (approx. 5 min), we can be generally concluded that no interference has occurred. However, 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. Identification of losses in sample handling and extraction may be identified by performing recovery experiments, i.e., by adding D-glucose to the sample in the initial extraction steps.

General information on sample preparation
To develop this assay use clear, colorless and practically neutral liquid samples directly, or after dilution; filter turbid solutions; degas samples containing carbon dioxide (e.g. by filtration); adjust acid samples, which are used undiluted for the assay, to pH 8 by adding sodium or potassium hydrosol; adjust acid and weakly colored samples to pH 8 and incubate for approx. 15 min; measure "colored" samples (if necessary adjusted to pH 8) against a sample blank; treat "strongly colored" samples that are used undiluted or with a higher sample volume with PVPP; crush or homogenize solid or semi-solid samples, extract with water or dissolve; extract samples containing fat with hot water.

Examples of sample preparation
Levels of D-glucose present in the cuvette should range between 4 and 80 μg in a sample volume of 0.10-2.00 ml.

Determination of D-glucose in milk
Introduce 20 mL of milk into a 100 mL volumetric flask and dissolve with 50 mL of distilled water. Make up to the mark, mix and filter. Discard the first 5 mL of the filtrate and use the clear, undiluted filtrate for the assay (0.1-2.0 mL).

Determination of D-glucose in fermentation samples and cell culture medium
Incubate approximately 10 mL of the solution at 90-95 °C for 10 min to inactivate most enzyme activities. Centrifuge or filter and use the supernatant or clear filtrate for the assay.

Solid foodstuffs
Plant materials should be milled to pass a 0.5 mm screen. Homogenize solid foodstuffs in a mixer, meat grinder or mortar. Weigh out a representative sample and extract with water (heated to 60 °C, if necessary). Quantitatively transfer to a volumetric flask and dilute to the mark with distilled water. Mix, filter and use the appropriately diluted, clear solution for the assay.

References

Recommendations
This method is recommended/approved by the:
- European Commission Regulation (analysis of wine);
- Association of Official Analytical Chemists (AOAC) (analysis of wine);
- Austrian, German, Italian and Swiss food laws;
- European, Dutch, French, German and Russian standards (EN, NEN, NF, DIN, GOST);
- International Wine Office (OIV), International Federation of Fruit Juice Producers (IUF); Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European Economic Community (A.I.J.N.), and Mitteleuropäische Brautechnische Analysenkommission (MEBAK) (Central European Commission for Brewing Technology).

Please enquire info@nzytech.com to obtain any additional information about this kit or other kits for the determination of “reducing sugars”.

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