AK0003_IFU_EN_V2302

D-Glucose HK, UV method

Catalogue number	Presentation
AK00031	110 tests (manual) / 1100 tests (microplate)

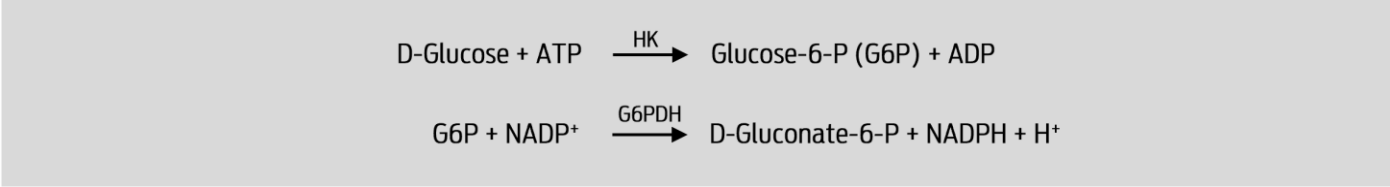
Application

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 foods, 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



The amount of NADPH formed through the combined action of hexokinase (HK) and glucose-6-P dehydrogenase (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 glucokinase 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 (12.5 mL, 2 M, pH 7.6), $MgCl_2$ (100 mM) and sodium azide (0.02 % w/v) as a preservative. Store at 2 °C to 8°C.

Solution 2. $NADP^+$ (150 mg) plus ATP (440 mg). Store at 2 °C to 8°C (Long term storage: -30 °C to -15 °C)

Dissolve in 12 mL of distilled water, divide into appropriately sized aliquots and store in PP tubes at -30 °C to -15 °C between use and keep cool during use.

Suspension 3. Hexokinase (EC 2.7.1.1) and glucose-6-P dehydrogenase (EC 1.1.1.49) in 3.2 M ammonium sulphate (2.25 mL). Store at 2 °C to 8°C. Swirl bottle before use.

Solution 4. D-Glucose standard solution (5 mL, 0.40 mg/mL) in 0.02% benzoic acid. This standard solution can be used when there is some doubt about the method accuracy. Store at 2 °C to 8°C.

Protocol (endpoint analysis)

Wavelength: 340 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: ~25 °C

Final volume: 2.32 mL

Sample solution: 4-80 µg of D-glucose per cuvette (in 0.10-2.00 mL sample volume)

Read against air (without a cuvette in the light path) or against water

PIPETTE TO CUVETTES (mL)	**BLANK**	**SAMPLE**
Distilled water (at ~25 °C)	2.10	2.00
Sample	-	0.10
Solution 1 (imidazole buffer)	0.10	0.10
Solution 2 ($NADP^+$ + ATP)	0.10	0.10
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

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 ε of NADH at 340 nm (6300 $L \times mol^{-1} \times cm^{-1}$), is calculated as follows:

$$C\ (D\text{-Glucose}) = 0.6634 \times \Delta A_{D\text{-Glucose}} \quad [g/L]$$

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.

Alternative procedures (micro-volumes)

Although this kit has been developed to work in cuvettes, it can be easily adapted for use in 96-well microplates or in auto-analysers. Basically, the assay volumes for the cuvette format have to be reduced approximately 10-fold for use in microplate format or in auto-analyser format. However, when using these micro-volume formats, you must be aware that the radiation pathlength is usually smaller than 1 cm, which is the standard cuvettes pathlength. Thus, to perform the calculation of the amount of analyte in the samples follow one of the three possible strategies described in the “Alternative Procedures”, available on the NZYtech website.

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

The amount of D-glucose present in the cuvette should range between 4 and 80 µg. Thus, if a sample volume of 0.10 mL is used the sample solution must be diluted to yield a D-glucose concentration between 40 and 800 mg/L. However, the sample volume can vary from 0.10 to 2.00 mL, by replacing water (analyte range from 2 to 800 mg/L).

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 hydroxide solution; 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

Determination of D-glucose in milk

Introduce 20 mL of milk into a 100 mL volumetric flask and add the following solutions: 10 mL of Carrez I solution (3.60 g of potassium hexacyanoferrate (II) in 100 mL of distilled water), 10 mL of Carrez II solution (7.20 g of zinc sulphate in 100 mL of distilled water) and 20 mL of NaOH solution (100 mM). Mix after each addition. Fill up to the mark with distilled water, mix and filter. Use 1.00 mL of the filtrate for the assay.

Determination of D-glucose in preserves, and other vegetable and fruit products

Precisely 10 g of the sample to be analyzed should be homogenized in a mixer. Introduce 0.5 g of the sample 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.

Determination of D-glucose in solid foodstuffs

Plant materials should be milled to pass a 0.5 mm screen. Homogenize solid foodstuffs in a mixer, meat grinder or mortar. A representative sample should be weighed and extracted 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

Kunst, A., Draeger, B. & Ziegenhorn, J. (1988). D-Glucose. In: Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), 3rd ed., Vol.VI, pp. 163-172, VCH Publishers (UK) Ltd., Cambridge, UK

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 (IFU), 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, including additional specific applications