AK0016_IFU_EN_V2303

D-Glucose GOD-POD, colorimetric method

Catalogue number	Presentation
AK00161	660 tests

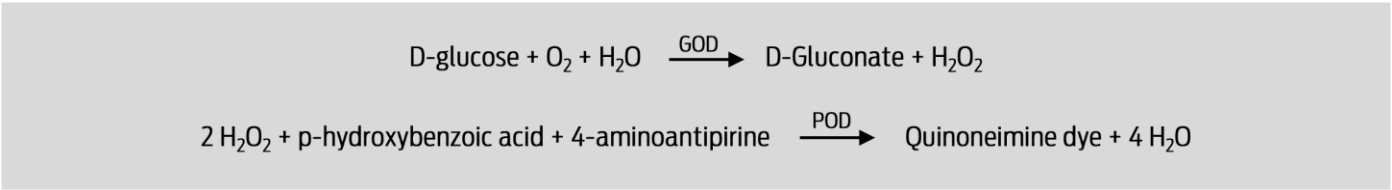
Application

This rapid and simple enzymatic colorimetric method is used for the quantitative 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 510 nm, is stoichiometric with the amount of D-glucose in sample volume.

Specificity

This method is specific for D-glucose since the kit employs high purity glucose oxidase and peroxidase.

Sensitivity and detection limit

The sensitivity of the assay is based on 0.010 AU and a sample volume of 0.10 mL. The detection limit is of 10 µg D-glucose per assay (v = 0.10 mL).

Linearity and precision

Linearity of the determination exists from 10 to 100 µg D-glucose per assay (v = 0.10 mL). The coefficient of variation is approx. 1 to 2 %.

Kit composition

Solution 1 (×2). GOD-POD reagent buffer (30 mL, pH 7.4), 0.42 M Potassium fosfate, 0.35 M p-hydroxybenzoic acid and sodium azide (0.64% w/v) as a preservative. Store at 2 °C to 8 °C.

Dilute the contents of bottle to 1 L with distilled water and use immediately. Do not dissolve the content of the second bottle until required.

Mixture 2 (×2). GOD-POD reagent enzymes. Freeze-dried powder of glucose oxidase (GOD), peroxidase (POD) and 4-aminoantipyrine. Store at 2 °C to 8 °C. (Long-term storage at -30°C to -15 °C)

Dissolve the contents of bottle *Mixture 2* in approx. 20 mL of *Solution 1* and quantitatively transfer this to the bottle containing the remainder of *Solution 1*. Cover this bottle with aluminum foil to protect the enclosed reagent from light. Stable for 3 months at 2 °C to 8 °C or 12 months at -30 °C to -15 °C. Do not dissolve the content of the second bottle until required.

Solution 3. D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid. Store at 2 °C to 8 °C.

Protocol (endpoint analysis)

Wavelength: 510 nm

Cuvette: 1 cm light path (glass or plastic)

Temperature: 40-50 °C

Final volume: 3.10 mL

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

Read against reagent blank

PIPETTE INTO CUVETTES (mL)	BLANK	STANDARD	SAMPLE
GOD-POD reagent	3.00	3.00	3.00
D-Glucose standard	-	0.10	-
Sample	-	-	0.10
Buffer or water	0.10	-	-
Mix, incubate at 40-50 °C for 20 min and read absorbances at 510 nm against the reagent blank to obtain ΔA_{sample} and $\Delta A_{D\text{-glucose standard}}$*			

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

Calculation

The concentration of D-glucose is calculated as follows:

$$C\ (D\text{-Glucose}) = \frac{\Delta A_{sample}}{\Delta A_{G\text{-Glucose standard}}} \quad [g/L]$$

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, 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 10 and 100 µg. Thus, the sample volume used (0.10 mL) implies that the sample solution must be diluted to yield a D-glucose concentration between 100 and 1000 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 0.1 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 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

Trinder, P. (1969). Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J. Clin. Pathol., 22, 2, 158-161.

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