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ProductInformation

Glucose (HK) Assay Kit

Product Code **GAHK-20**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industry. Enzymatic methods are specific, reproducible, sensitive, rapid, and therefore, ideal for analytical purposes. Due to the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation. This kit is for the quantitative, enzymatic determination of glucose in food and other material.

Principle

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-Phosphate} + \text{ADP}$$

$$\text{G6P} + \text{NAD} \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate} + \text{NADH}$$

Glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase. Glucose-6-phosphate (G6P) is then oxidized to 6-phospho-gluconate in the presence of oxidized nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH. The consequent increase in absorbance at 340 nm is directly proportional to glucose concentration.

Components

1. Glucose (HK) Assay Reagent
   (Product Code G 3293)
   Reconstitute the vial contents with 20 ml of water. After addition of water, stopper the vial and immediately mix several times by inversion. DO NOT SHAKE.

   Each vial when reconstituted with 20 ml of water contains 1.5 mM NAD, 1.0 mM ATP, 1.0 unit/ml of hexokinase, and 1.0 unit/ml of glucose-6-phosphate dehydrogenase with sodium benzoate and potassium sorbate as preservatives.

   The dry reagent is stored at 2-8 °C. The reagent should be discarded if the vial contents exhibit caking due to possible moisture penetration, if the vial contents do not dissolve completely upon reconstitution, or if the reconstituted solution appears turbid.

   The reconstituted reagent is stable, in the absence of visible microbial growth for 7 days at 18-26 °C and for at least 4 weeks at 2-8 °C. The reagent is not suitable for use if the absorbance of the freshly reconstituted solution measured at 340 nm versus water as the reference is greater than 0.350.

2. Glucose Standard Solution
   (Product Code G 3285)
   D-Glucose, 1.0 mg/ml in 0.1% benzoic acid. This standard is **traceable to an NIST standard** and is supplied ready-to-use. It is stable at 2-8 °C for at least six months. Discard if turbidity develops.

Equipment Required but Not Provided

1. Spectrophotometer suitable for measuring absorbance at 340 nm.
2. Cuvets
3. Pipettes capable of accurately dispensing 10 µl to 1 ml.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Sample Preparation:

Liquids - Dilute sample with deionized water to 0.05 - 5 mg of glucose/ml.

Filter or deproteinize solution if necessary to clarify. Solutions that are strongly colored and that have a low glucose concentration should be decolorized. Carbonated or fermented products must be degassed.

Solids - Weigh out sample to nearest 0.1 mg. Extract sample with deionized water. The solution may be heated (<75 °C) to aid extraction. Dilute with deionized water to 0.05 – 5 mg of glucose/ml. Filter or deproteinize solution, if necessary, to clarify.

Determination:

Pipette a volume of solution corresponding to 0.5 - 50 µg of glucose. Repeat assay and vary the sample volume, if necessary, to give an ΔA_{340} between 0.03 and 1.6.

1. Pipette the following solutions into the appropriately marked test tubes.

Tube	Glucose Assay Reagent (ml)	Sample Volume (µl)	Volume of Deionized Water (ml)
Sample Blank	---	Same as for Test	1.0
Reagent Blank	1.0	---	Same as Sample Volume for Test
Test	1.0	10 - 200	---

2. Mix tubes and incubate for 15 minutes at room temperature (18-35 °C).

3. Measure the absorbance at 340 nm versus deionized water.

Calculations:

The total blank must take into account the contribution to the absorbance of the sample and the glucose assay reagent.

$$A_{\text{Total Blank}} = A_{\text{Sample Blank}} + A_{\text{Reagent Blank}}$$

$$\text{mg glucose/ml} = \frac{(\Delta A)\,(TV)\,(\text{Glucose Molecular Weight})\,(F)}{(\varepsilon)(d)(SV)(\text{Conversion Factor for } \mu g \text{ to mg})}$$

$$\text{mg glucose/ml} = \frac{(\Delta A)\,(TV)\,(180.2)\,(F)}{(6.22)\,(1)\,(SV)\,(1{,}000)}$$

$$\text{mg glucose/ml} = \frac{(\Delta A)\,(TV)\,(F)\,(0.029)}{(SV)}$$

$\Delta A = A_{Test} - A_{Total\ Blank}$
TV = Total Assay Volume (ml)
SV = Sample Volume (ml)
Glucose MW = 180.2 g/mole or equivalently 180.2 µg/µmoles
F = Dilution Factor from Sample Preparation
ε = Millimolar Extinction Coefficient for NADH at 340 nm
Millimolar $^{-1}$ cm $^{-1}$ or equivalently (ml/µmoles)(1/cm)
d = Light path (cm) = 1 cm
1,000 = Conversion Factor for µg to mg

References

1. Bondar, R.J.L., and Mead, D.C., Clin. Chem. **20**, 586-590 (1974).
2. Kunsst, A., *et al*., Methods of Enzymatic Analysis, 3rd Edition, Bergmeyer, H.U., ed., Academic Press (New York, NY: 1984) Volume 2, 163-172.
3. Southgate, D.A. T., Determination of Food Carbohyrates, Applied Science Publishers (London, UK: 1976).

CMH/MAM 9/04