ProductInformation

GLUCOSE (HK) ASSAY KIT
Kit GAHK-20
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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:

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

$$\text{Glucose-6-Phosphate} + \text{NAD} \xrightarrow[\text{Dehydrogenase}]{\text{Glucose-6-Phosphate}} \text{6-Phosphogluconate} + \text{NADH}$$

Glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase. Glucose-6-phosphate is then oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase. 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.

APPARATUS:

1. Spectrophotometer suitable for measuring absorbance at 340 nm.

2. Cuvets

3. Pipets capable of accurately dispensing 10 µl to 1 ml.

REAGENTS:

Refer to Material Safety Data Sheets for updated risk, hazard or safety information.

1. **Glucose (HK) Assay Reagent** (Sigma Product No. G2020)

   Reconstitute reagent vial with 20 ml of deionized water. Stopper vial and immediately mix several times by inversion. DO NOT SHAKE.

   Each vial when reconstituted with 20 ml of deionized water contains 1.5 mM NAD, 1.0 mM ATP, 1.0 U/ml Hexokinase and 1.0 U/ml of Glucose-6-Phosphate Dehydrogenase, and sodium azide, 0.05%, as preservative.

   The dry reagent is stored at 2-8°C. Reconstituted reagent is stable, in the absence of visible microbial growth for 7 days at 18-26°C and for 4 weeks at 2-8°C. Reagent is not suitable for use if the absorbance of the freshly reconstituted solution measured at 340 nm vs water as reference is greater than 0.350. Reagent should be dicarded if the vial exhibits caking due to possible moisture penetration, if the vial contents do not dissolve completely upon reconstitution or if the solution appears turbid.

2. **Glucose Standard Solution** (Sigma Product No. G3285)

   D-Glucose, 1.0 mg/ml in 0.1% benzoic acid. Supplied ready to use. Solution is stable at 2-8°C for at least six months. Discard if turbidity develops.

SAMPLE PREPARATION:

Liquids Dilute sample with deionized water to approximately 0.05 - 5 mg 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 approximately 0.05 - 5 mg glucose/ml. Filter or deproteinize solution if necessary to clarify.

DETERMINATION:

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

1. Pipet 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.

CALCULATIONS:

$A_{\text{TOTAL BLANK}} = A_{\text{SAMPLE BLANK}} + A_{\text{REAGENT BLANK}}$

$\Delta A = A_{TEST} - A_{TOTAL\ BLANK}$

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

$$= \frac{(\Delta A)(TV)\,(180.2)\,(F)}{(6.22)\,(1)\,(SV)\,(1000)}$$

$$= \frac{(\Delta A)\,(TV)\,(F)\,(0.029)}{(SV)}$$

ε = Millimolar Extinction Coefficient for NADH at 340 nm
d = Light path (cm)
TV = Total Assay Volume
SV = Sample Volume
F = Dilution Factor from sample preparation

REFERENCES:

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2. Kunsst, A., Draeger, B. and Ziegenhorn, J., Methods of Enzymatic Analysis, H.U. Bergmeyer, Ed., New York, Academic Press, 3rd Edition, Volume 2, 163-172 (1984).

3. Southgate, D.A. T., Determination of Food Carbohyrates, Applied Science Publishers, London (1976).