

Glucose Reagent

(HK)

Catalog #: 43714

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of Glucose in serum.

SUMMARY AND EXPLANATION ¹

Glucose measurements are used in the diagnosis and treatment of many metabolic diseases. Elevated glucose levels (hyperglycemia) may be seen in patients with diabetes mellitus, diuretic therapy, severe stress, and cerebrovascular accidents. Decreased glucose levels (hypoglycemia) may be seen in insulinoma, insulin administration, hypothyroidism, and severe liver disease.

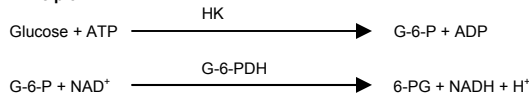
METHODOLGY

There are a large number of methods in existence for the measurement of glucose in biological fluids. Early methods such as the Folin-Wu² and Somogyi-Nelson³ depended on the reduction of heavy metals by the aldehyde group of glucose. These methods are subject to interference by carbohydrates other than glucose. The ortho-toluidine method, introduced in 1959⁴ and later modified^{5,6} to react directly with serum, is specific for aldoses but uses a strong, noxious, corrosive acid requiring incubation at elevated temperatures.

Enzymatic methods were first described in the 1940's⁷ with varied modifications described to date.^{8,9}

The SDI Biomed glucose hexokinase method is based on a modification of Slein¹⁰, using hexokinase and glucose-6-phosphate-dehydrogenase to catalyze the reaction. The method is also based on the reference method proposed by the FDA for measuring glucose.

Principle



Glucose is phosphorylated with adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase (HK). The product, glucose-6-phosphate (G6P) is then oxidized with the concomitant reduction of nicotinamide adenine dinucleotide (NAD) to NADH in the reaction catalyzed by glucose-6-phosphate-dehydrogenase (G6PDH). The formation of NADH causes and increase in absorbance at 340 nm. The increase is directly proportional to the amount of glucose in the sample.

REAGENT COMPOSITION

Active Ingredients	Concentration
ATP	2.1 mM
NAD	2.5 mM
Hexokinase (yeast)	>1500 U/L
G-6-PDH (L.m.)	>3200 U/L
pH 7.5 ± 0.1	
Nonreactive stabilizers and fillers.	

Precautions:

1. This reagent is for *in vitro* diagnostic use only.
2. Reagent contains Sodium Azide (0.02%) as a preservative. In a dry state may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

REAGENT PREPARATION

Reagent comes in a ready to use form.

REAGENT STORAGE

1. The reagent should be stored refrigerated at 2-8°C.
2. The reagent is stable until the expiration date when stored at 2-8°C.

REAGENT DETERIORATION

Do not use if:

1. Reagent has an absorbance greater than 0.50 when measured against water at 340 nm.
2. The reagent fails to recover stated control values or meet stated linearity.
3. The reagent develops turbidity, indicating contamination.

SPECIMEN COLLECTION AND STORAGE

Serum: Use fresh, unhemolyzed serum removed from the clot as soon as possible..
Glucose in serum is stable for 3 days refrigerated at 2-8°C.¹²

INTERFERENCES

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference (± 10%) from hemoglobin up to 400 mg/dL.

Bilirubin:

No significant interference (± 10%) from bilirubin up to 25.8 mg/dL.

Lipemia:

No significant interference (± 10%) from lipemia up to 396 mg/dL measured as triglycerides.

A number of drugs and substances may affect the accuracy of this test. See Young, et al¹³

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. A SDI CA480 Clinical Chemistry System.
2. Deionized water and related equipment, e.g.: pipettes
3. Analyzer specific consumables, e.g.: sample cups
4. Control, and Calibrator materials such as those provided by SDI Biomed.

ASSAY PROCEDURE

System Parameters

Glucose Hexokinase (Liquid)

TEMPERATURE:	37°C
WAVELENGTH:	340 nm
ASSAY TYPE:	Endpoint
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 100
e.g. Sample Vol.	0.003 mL (3mL)
Reagent Vol.	0.300 mL (300 mL)
INCUBATION TIME:	3 Min

Procedure Note:

1. Samples with glucose above 650 mg/dL should be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
2. The reagent and sample volumes may be altered proportionally to accommodate various instrument requirements.

Calculations:

(A = Absorbance)

$$\frac{A(\text{patient})}{A(\text{standard})} \times \text{Concentration of standard} = \text{Glucose (mg/dL)}$$

Example:

$$\begin{array}{l} A(\text{patient}) = 0.10 \\ A(\text{standard}) = 0.300 \\ \text{Concentration of standard} = 238 \text{ mg/dL} \end{array}$$

$$\frac{0.10}{0.30} \times 238 = 79 \text{ mg/dL Glucose}$$

Limitations:

1. Samples with glucose above 650 mg/dL should be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
2. Extremely hemolyzed samples should not be used for the glucose assay.

CALIBRATION

Use an aqueous Glucose standard, or an appropriate serum calibrator.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Glucose values

EXPECTED VALUES ¹¹

Normal range is reported to be: 70-105 mg/dL.

It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE

Linearity:

When run as recommended the assay is linear to 650 mg/dL

Method Comparison:

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	42
Range of samples:	7 – 593 (mg/dL)
Correlation Coefficient:	0.9992
Slope:	0.9456
Intercept:	7.6(mg/dL)

Precision:

Within Run n=40	Level 1	Level 2	Level 3
Mean (mg/dL)	58.3	114.1	348.9
S.D. (mg/dL)	0.8	0.9	3.0
C.V. (%)	1.3	0.8	0.9

Total

n=40 (10 days / 2 runs per day / 2 replicates per run)

Mean (mg/dL)	58.3	114.1	348.9
S.D. (mg/dL)	1.2	2.3	4.7
C.V. (%)	2.0	2.0	1.3

Sensitivity:

A calibration factor of approximately 550 was obtained, which is equivalent to a sensitivity of 0.0018 D Abs per mg/dL.

REFERENCES

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