Glucose-HK

Hexokinase. Enzymatic – UV

Quantitative determination of glucose

IVD

PACKAGING

Ref.: 101-0366	Cont.: 12 x 50 mL
Ref.: 101-0391	Cont.: 4 x 50 mL

Store at 2 - 8º C

CLINICAL SIGNIFICANCE

Glucose is a major source of energy for most cells of the body; insulin facilitates glucose entry into the cells.

Diabetes is a disease manifested by hyperglycemia; patients with diabetes demonstrate an inability to produce insulin[1,5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Hexoquinase (HK) catalyzes the phosphorylation of glucose to glucose-6-phosphate (G6P) by ATP. The formed glucose-6-phosphate is reduced to 6-phosphogluconate in the presence of glucose-6-phosphate dehydrogenase (G6P-DH) with the subsequent reduction of NAD to NADH:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{G6P} + \text{NAD}^+ \xrightarrow{\text{G6P-DH}} \text{6-Phosphogluconate} + \text{NADH} + \text{H}^+$$

The increase in concentration of NADH is proportional to the glucose concentration in the sample[1,2].

REAGENTS

R 1 Buffer	TRIS pH 7.5	4 mmol/L
	ATP	2.1 mmol/L
	Mg^{2+}	0.8 mmol/L
R 2 Enzymes	NAD^+	2 mmol/L
	Hexoquinase (HK)	1000 U/L
	Glucose-6-phosphate (G6P-DH)	1000 U/L
GLUCOSE CAL	Glucose aqueous primary standard 100 mg/dL	

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PREPARATION

Working reagent (WR): Dissolve (→) the contents of one vial of R 2 Enzymes in one bottle of R 1 Buffer.

Cap and mix gently to dissolve contents.

The reagent is stable after reconstitution 1 month in the refrigerator (2 - 8º C) or 7 days at room temperature (15 - 25º C).

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm ≥ 0.30.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

- Serum or plasma free of hemolysis[1].
  Serum should be removed from the clot as quickly as possible.
- Urine[1].

Stability of the sample: Glucose in serum or plasma is stable at 2 - 8º C for 3 days.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

GLUCOSE CAL: Proceed carefully with this product because due its nature it can get contamined easily.

Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . 1 cm. light path
   Temperature. . . . . 37º C / 15 - 25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Standard[(Note 1-2)] (µL)	--	10	--
Sample (µL)	--	--	10

4. Mix and incubate for 5 min. at 37º C or 10 min. at room temperature (15 - 25º C).
5. Read the absorbance (A) of the samples and calibrator, against the Blank.

CALCULATIONS

$$\frac{(A)Sample - (A)Blank}{(A)Standard - (A)Blank} \times 100\ (\text{Calibrator conc.}) = \text{mg/dL glucose in the sample}$$

Conversion factor: mg/dL x 0.0555= mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma:

60 – 110 mg/dL ≅ 3.33 – 6.10 mmol/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 0.16 mg/dL to *linearity limit* of 600 mg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

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Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	99.5	244	98.0	247
SD	0.83	1.70	1.60	3.75
CV (%)	0.83	0.70	1.63	1.51

Sensitivity: 1 mg/dL = 0.0036 A.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.9891
Regression equation: y=9877x + 0.9366
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES
Hemoglobin up to 19 g/L and bilirubin up to 100 mg/L, do not interfere[1].
A list of drugs and other interfering substances with glucose determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY
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