Glucose Estimation Kit

High-Q Glucose - ML

(GOD - PAP Trinder's Method)

Command on Quality

Intended Use
Kit for the quantitative determination of Glucose in Human Serum and Plasma

Summary and Clinical Significance:
Glucose is the central energy source of the cells in the organism. The most common supply follows hydrolytic cleavage of polymeric carbohydrates, in general starch. Glucose is a monosaccharide with a postprandial concentration of 5 mmol/l in the blood and serves as an indispensable energy-supply for cellular functions. The glucose catabolism takes place via the glycolysis as the first step, followed by the citric acid cycle and oxidative phosphorylation. Glucose regulations become executed the diagnosis and course control of carbohydrate metabolism illnesses like the diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia and with insulinoma. The test bases on the coupling of the enzymatic oxidation of glucose by glucose oxidase resulting in hydrogen peroxide, which is subsequently used for the generation of a coloured product by peroxidase. In the Trinder method the carcinogenic ortho-dianisidine used in earlier formulations has been replaced by phenol and 4-amino-antipyrine.

Principle:

$$Glucose + O_2 + H_2O \xrightarrow{GOD} Gluconic\ Acid + H_2O_2$$

$$H_2O_2 + Phenol + 4\text{-}Aminoantipyrene \xrightarrow{POD} Red\ quinoneimine\ Complex + H_2O$$

Normal Range:

Serrum / Plasma	:	70 - 110 mg/dl (Fasting)
		70 - 140 mg/dl (Post Prandial)
CSF	:	40 - 70 mg/dl

It is recommended that laboratories should establish their own normal range.

Storage and stability:
All the reagents are to be stored at 2-8^{0}C and are stable till the expiry date mentioned on the labels when properly stored.

Specimen collection and preservation:

Serum/Plasma is the preferred specimen.

Blood should be collected in a clean dry container. Serum or plasma should be separated from the cells at the earliest possible (within 30 minutes), as the rate of glycolysis is approximately 7 mg% per hour at room temperature.

For plasma separation following anticoagulants may be used.

EDTA	2 mg/mL of blood
CITRATE	6 mg/mL of blood
HEPARIN	200 IU/mL of blood
OXALATE	3 mg/mL of blood
SOD. FLUORIDE	10 mg/mL of blood

Sodium Fluoride is preferred as anticoagulant due to its anti glycolytic activity. Higher concentration of Sodium fluoride i.e. more than 10 mg/ml blood should be avoided as it may inhibit the colour development. Glucose is stable for 24 hours in neatly separated plasma and serum.

If the estimation is not possible within 24 hours then the specimen should be preserved at -10° C and should be used within 30 days.

Reagent Presentation:
High-Q Glucose - ML is available as Single Liquid Reagent (Ready-to-Use) and is stable till the expiration date mentioned on the labels when stored at 2-8^{0}C. When opened, contamination must be avoided.

Quality Control:
To ensure adequate Quality Control, the use of commercial reference control serum is recommended with each assay batch. Use of Quality control material checks both, the instrument and the reagent performances.

Procedure: End Point Method:
System Parameters:

Reaction Type	:	End-Point
Reaction Time	:	10 mins. at 37° C/15 mins. at R.T
Wavelength	:	505 nm. (490 - 550 nm.)
Zero Setting with	:	Reagent Blank
Sample Volume	:	10 µl
Reagent Volume	:	1.0 ml
Standard Concentration	:	100 mg/dl
Linearity	:	500 mg/dl

Pipette into three test tubes labeled Blank (B), Standard (S) Test (T) as follows:

Reagent	For 1 ml Procedure		
	B	S	T
Glucose Reagent	1.0 ml	1.0 ml	1.0 ml
Glucose Standard (Conc. 100 mg/dl)	----	10 µl	----
Specimen	----	----	10 µl

Mix and incubate for 10 minutes at 37^{0}C or 15 minutes at R.T.

Mix and read absorbances of Standad (S) and Test (T) against Reagent Blank (B) at 505 nm (490-550 nm).

Calculations:

$$\text{Glucose Conc. in mg/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times 100$$

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Test Procedure: (2 Point Kinetic Method) :

System Parameters

Reaction Type	2 Point Kinetic (Fixed Time)
Reaction Direction	Increasing
Flow Cell Temperature	37° C
Blanking With	Distilled Water
Delay Time	20 Sec
Measuring Time	40 Sec
Wavelength	505 nm. (490 - 550 nm.)
Sample Volume	10 µl
Reagent Volume	1.0 ml
Standard Concentration	100 mg/dl
Linearity	1000 mg/dl

Bring the High-Q Glucose - ML Reagent to R.T. before use. Pipette into test tubes labeled Standard (S) and Test (T) as follows :

Reagent	S	T
Glucose Reagent	1.0 ml	1.0 ml
Glucose Standard (conc. 100 mg/dl)	10 µl	--
Specimen	--	10 µl

Mix well and immediately aspirate in to the analyzer. Read absorbances of Standard (S) and Test (T) against distilled water at 505 nm as follows :

Initial absorbance A_0 = Exactly after 20 Sec.
Final absorbance A_1 = Exactly 40 Sec. After A_0

Determine △Abs for S and T

△ Abs for S = Abs S_1 - Abs S_0
△ Abs for T = Abs T_1 - Abs T_0

CALCULATIONS :

GLUCOSE conc. In mg/dl = $\frac{\triangle \text{Abs for T}}{\triangle \text{Abs for S}}$ X 100

Notes:

1. Contamination of Standard and Reagent must be avoided. After use, all the reagents must be immediately stored back at 2-8°C.
2. Replug the Glucose Standard vial after use. Use clean glassware / microtips while pipetting Glucose standard.
3. If a larger volume of reagent is required for absorbance reading, requisite volumes can be taken in multiples keeping the same ratio of reagent to specimen / Standard.
4. For sample values higher than 500 mg/dl, dilute the sample with normal saline and multiply the result with appropriate dilution factor.
5. Programmes for specific autoanalyzers are available on request.

References:

1. Trinder, P. (1969) Annals. Clin. Bio Chem. 6, 24.
2. Bergmayer, H.V. (1974) Method of Enzymatic Analysis., P. 1196.

Ordering Information

Ref./Cat. No.	Pack Size	Presentation
P-GLU - 600	6 x 100 ml	Mono Reagent
P-GLU - 1000	4 x 250 ml	

Product Features

- **Liquid Stable, Ready to use Mono Reagent**
- **End Point and Fixed time procedures**
- **10 Minutes End Point Assay**
- **1 Minute Fixed Time Assay (20 Sec Delay+40 Sec Measuring)**
- **Linearity : 500 mg/dl**
- **Lipid Clearing Factor**
- **Measuring Wavelength 505 nm (490 – 550 nm)**
- **Aqueous Glucose Standard provided (Standard Conc: 100 mg/dl)**
- **Serum / Heparinized or EDTA Plasma as Specimens**
- **Available as multipurpose reagents and dedicated system packs**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

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Manufactured in India by :
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