GLUCOSE (10 Min.)

(GOD/POD, Enzymatic)

ENZOPAK

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Ref. CC2-GLU.11M, 10x100 ml
CC2-GLU.11MU, 10x500 ml
CC2-GLU.11MV, 5x100 ml

INTENDED USE

Reagent kit for quantitative estimation of glucose in serum or plasma.

PRODUCT HIGHLIGHTS

- Low blank.
- Formulations suitable for every need.
  a) Two reagents with long stability.
  b) Pack sizes suitable to every need.
- Linearity and accuracy as per international standard.

INTRODUCTION

Conventional methods like ferricyanide, Nelson-Somogyii, Folin - Wu, O-Toludine involved lengthy procedures and were cumbersome. Some of these methods involved steps like precipitation, filteration and boiling. These methods have drawbacks, are non-specific, imprecise, insensitive and inaccurate.

This estimation of Glucose is based on GOD/POD method (Glucose oxidase / Peroxidase) as described by Trinder and is specific and accurate. The method selectively estimates ß -D Glucose in serum or plasma.

DIAGNOSTIC SIGNIFICANCE

Blood glucose estimations are mainly carried out for the diagnosis and follow up of diabetes mellitus.

Glucose circulating in blood plasma is in dynamic equilibrium with absorption of carbohydrates from intestine, high levels of Glucose induces glycogen synthesis and low levels generate carbohydrates, in turn, glucose. Similar pathways are also encountered with Fatty acids and Amino acids (protein). Deficiency of insulin-like activity is a condition resulting in hypoglycemia. Hyperglycemia and Hypoglycemia are also associated with various hormonal disorderes e.g. Pituitary, Thyroid etc.

PRINCIPLE

Glucose oxidase oxidise the specific substrate, ß -D-glucose, to gluconic acid and hydrogen peroxide is generated. Hydrogen-peroxide thus produced is acted upon by peroxidase and oxygen is liberated. The liberated oxygen is transferred to chromogen system consisting of 4 aminoantipyrine and phenolic compound to produce red quinoneimine dye. The intensity of colour is directly proportional to the concentration of glucose and is measured photometrically at 505 nm (505-540 nm or with GREEN filter).

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + \text{Phenolic Compound} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Coloured Complex} + H_2O$$

PRESENTATION

Store all reagents at 2-8^{0}C	No. of Bottles 5x100ml	10x100ml	10x500ml
• 1 Glucose (Enzyme/Chromogen)	5	10	10
• 2 Glucose (Buffer)	1	1	1
• Standard (100 mg/dl)	1	1	3
• Reconstitution Bottle	1	1	1

2 Glucose Buffer (Ready to use) is provided separately.
For 10x500 ml Reconstitution Bottle provided separately.

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• Buffer	100 mmol/L
• GOD	≥15000 U/L
• POD	≥ 1000 U/L
• 4 – AAP	0.2 mmol/L

pH 7.4 ± 0.5 at 25^{0}C
AQUA Buffer
Glucose Standard (100 mg/dl)
Also contains non-reactive fillers and Stabilizers.

PRECAUTION

Glucose is for *IN-VITRO* diagnostic use only.
Reagent Contains Sodium Azide. DO NOT INGEST.

PREPARATION OF WORKING REAGENT

For 10 x 100 ml & 5 x 100 ml

Transfer contents (powder mixture of Enzyme/Chromogen) of one vial of 1 Glucose to the bottle provided for reconstitution. To this add 100 ml of 2 Glucose AQUA Buffer mix well to dissolve. The reagent is now "Working Reagent" ready for use. Store at 2-8^{0}C when not in use.

For 10 x 500 ml

Transfer contents (powder mixture of Enzyme/Chromogen) of one vial of 1 Glucose to the bottle provided for reconstitution. To this add 500 ml of 2 Glucose AQUA Buffer mix well to dissolve. The reagent is now "Working Reagent" ready for use. Store at 2-8^{0}C when not in use.

REAGENT STORAGE AND STABILITY

1 Glucose (Powder/tablet)	2-8^{0}C	Until Expiry
2 Glucose (Aqua Buffer)	R.T.	Until Expiry
Glucose Standard (100 mg/dl)	2-8^{0}C	Until Expiry
Working Reagent (Protected from light)	2-8^{0}C	50 days

SPECIMEN COLLECTION

Blood sample collected with any one of the anticoagulants like flouride, oxalate, EDTA, heparin or without any of the anticoagulants can be used. As soon as the sample is collected, separate serum or plasma to prevent glycolysis.

REACTION PARAMETERS

- Type of Reaction : End Point
- Wavelength : 505 nm (500-540 nm)
- Flowcell Temperature : 37^{0}C
- Incubation : 10 min. at 37^{0}C
- Std. Concentration : 100 mg/dl
- Sample Volume : 10 µl (0.01 ml)
- Reagent Volume : 1.0 ml
- Zero setting with : Reagent Blank
- Light Path : 1.0 cm

TEST PROCEDURE

Pipette Into Test Tubes	Blank	Standard	Test
Working Reagent (ml)	1.0	1.0	1.0
Standard (ml)	-	0.01	-
Sample (ml)	-	-	0.01

Mix well and allow to stand for 10 Min. at 37^{0}C. Mix & read absorbance of standard and test at 505 nm (500-540 nm or with GREEN filter) against reagent blank.

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STABILITY OF FINAL REACTION MIXTURE

The colour of reaction mixture is stable for 2 hours at room temperature, when protected from direct light.

TEST RESULTS

$$\text{Glucose Concentration (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$

REACTION PARAMETERS

- Type of Reaction : Fix time/two point/initial rate
- Wavelength : 505 nm (500-540)
- Flowcell Temperature : 37^{0}C
- Delay Time : 30 seconds
- Interval : 60 seconds
- Measuring time : 90 seconds
- No. of reading : 2
- Standard/Sample volume : 10 µl (0.01 ml)
- Std. Concentration : 100 mg/dl
- Reagent Volume : 1.0 ml
- Zero setting with : Distilled water
- Light Path : 1.0 cm

TEST PROCEDURE

Pipette Into Test Tubes	Standard	Test
Working Reagent (ml)	1.0	1.0
Standard (ml)	0.01	-
Sample (ml)	-	0.01

Mix and aspirate. Record the absorbance of Standard (ST) and Test (TS) at 30 seconds (ST_1, TS1) and again at 90 seconds (ST_2, TS2) at 505 nm, against distilled water.

TEST RESULTS

$$\text{Glucose concentration (mg/dl)} = \frac{(TS_2 - TS_1)}{(ST_2 - ST_1)} \times 100$$

To convert mg/dl to mmol/lit. use the following factor.

1 mmol / lit. = 18 mg/dl
1 mg/dl = 0.056 mmol/lit

LIMITATIONS FOR INTERFERENCE

As per studies carried out for interference. Following results were obtained.

1. No Interference from Hemoglobin upto 187.5 mg/dl.
2. No Interference from free Bilirubin upto 25.0 mg/dl.
3. No Interference from Lipemic (Measured as Triglycerides) upto 1000 mg/dl.

NORMAL VALUES

Fasting: 70-110 mg/dl (3.90-6.11 mmol/lit)
Two Hours Post prandial: upto 140 mg/dl (7.78 mmol/lit)

LINEARITY

This method is linear upto 500 mg/dl. For sample value above 500 mg/dl, dilute the sample suitably with 0.9 % saline & repeat the assay. Apply correction due to dilution to arrive at a final result.

REFERENCES

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