Eco-Pak GLUCOSE

INTRODUCTION

1. *Eco-Pak* Glucose is a reagent set for determination of **True Glucose, based on enzymatic method** using Glucose oxidase and Peroxidase.
2. *Eco-Pak* Glucose has **one step reconstitution.** It involves the mixing of Enzyme and Diluent.
3. *Eco-Pak* Glucose is **linear** upto 450 mg%.
4. *Eco-Pak* Glucose is a **High Stability Reagent.**
5. *Eco-Pak* Glucose can be used on any **Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.
6. *Eco-Pak* Glucose can be determined in just **15 minutes** at 37°C or **30 minutes** at R.T. (25-30°C).
7. **Sodium Fluoride** (as an anticoagulant upto 10 mg/ml blood) does not affect glucose assay.
8. The influence of **Ascorbate, Bilirubin, Antidiabetic drugs and Haemoglobin** is negligible.
9. The reagent can also determine glucose using whole blood (0.02 ml) **collected from finger-prick.** The procedure is available upon request.

PRINCIPLE

Glucose oxidase (GOD) converts glucose to gluconic acid. Hydrogen peroxide formed in this reaction, in presence of peroxidase (POD), oxidatively couples with 4-aminoantipyrine and phenol to produce red quinoneimine dye. This dye has absorbance maximum at 505 nm. (500 - 550 nm.). The intensity of the colour complex is directly proportional to the concentration of glucose in specimen.

$$\beta\text{-D Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Giuconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Red dye} + H_2O$$

PREPARATION OF WORKING SOLUTION

Reconstitute enzyme & diluent as per instruction indicated on individual bottle label to prepare working solution. Mix by gentle swirling or inversion. DO NOT SHAKE VIGOROUSLY.

REAGENT STORAGE & STABILITY

Enzyme and Standard should be stored at 2-8°C. Diluent should be stored below 30°C and away from direct light.

The working solution is stable for 60 days when stored at 2-8°C (DO NOT FREEZE). The working solution should be stored in the dark bottle (working solution container) provided. This is critical because the reagent is light sensitive (auto oxidation of chromogen system by light and air), it should therefore be kept away from direct light.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Phosphate Buffer : pH 7.0	120 mmol/l
• Glucose oxidase	≥ 5000 IU/l
• Peroxidase	1050 IU/l
• 4-aminoantipyrine	0.20 mmol/l
• Phenol	11 mmol/l
• Stabilizers and inactive ingredients	

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Avoid the use of plastic or siliconized containers for blood collection which may prolong the clotting time. 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 (25-30°C).

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
• SODIUM FLUORIDE	10 mg/ml of blood

Sodium Fluoride is preferred as anticoagulant due to its antiglycolytic 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.

GL-2009-03-00I

PROCEDURE

- ☐ **Reaction type** ..... **End-Point**
- ☐ **Reaction time** .... **15 mins. at 37°C/30 mins. at R.T. (25 - 30°C)**
- ☐ **Wavelength** ..... **505 nm. (500-550 nm.)**
- ☐ **Zero setting with** ..... **Working Solution**
- ☐ **Blank absorbance limit** ..... **< 0.300 Abs.**
- ☐ **Sample volume** ..... **0.01 ml (10 µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Standard concentration** ..... **100 mg%**
- ☐ **Linearity** ..... **450 mg/dl**

Manual assay procedure

Prewarm at room temperature (25 - 30°C) the required amount of working solution before use. Perform the assay as given below :

1.0 ml procedure

	Serum / Plasma	Standard	Blank
	0.01 ml	0.01 ml	—
Working solution	1.0 ml	1.0 ml	1.0 ml

Incubation

Incubate the assay mixture for 15 minutes at 37°C or 30 minutes at room temperature (25 - 30°C). After completion of incubation period measure the absorbance against blank at 505 nm. Final colour is stable for two hours if not exposed to direct light.

Calculation :

$$\text{Glucose in mg\%} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 100$$

NOTE :

1. The specimen to working solution ratio can be altered proportionally without affecting the results.
2. The assay can also be performed using whole blood as specimen (0.02 ml). Procedure is available upon request.

EXPECTED VALUES

Fasting Blood Glucose	:	**60 to 110 mg%**
Postprandial Blood Glucose	:	**<145 mg%**

NOTE:

Expected range varies from population to population therefore each laboratory should establish its own normal range.

The whole blood glucose value is generally 10 to 15 percent lower than the serum or plasma glucose values due to the cell stroma.

No significant difference exists between capillary and intravenous blood glucose value, except at the peak of the Glucose Tolerance Test (GTT) where capillary blood glucose value is approximately 20% higher.

PROCEDURE LIMITATIONS

1. Discard the working solution if the absorbance is more than 0.300 against distilled water at 505 nm.
2. If the glucose value exceeds linearity limit then dilute specimen suitably with normal saline and repeat the assay. In such case the assay value should be multiplied with the dilution factor to obtain correct glucose value of the specimen.

QUALITY CONTROL

To ensure adequate quatity control, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use to quality control material checks both instrument and reagent functions together. Factors which might effect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

1. Trinder P., ***Annals. Clin. Biochem.*** 6,24 (1969).
2. Young,D. S. et al, ***Clinical Chemistry*** 21, 1 D (1975).
3. Bergmayer,H. V., ***"Methods of Enzymatic Analysis"***, A.P., N.Y. (1974). page 1196.

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

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