# Glucose Hexokinase Test Kit

Enzymatic Method

**Quantitative determination of Glucose Hexokinase in human Serum / Plasma. Only for *In Vitro* Diagnostic use**

**ORDER INFORMATION**

| REF | Pack Size |
|---|---|
| GHK 25 | 1 X 25 ml |
| GHK 50 | 1 X 50 ml |
| GHK 100 | 1 X 100 ml |
| GHK 1000 | 1 X 1000 ml |
| GHK 5000 | 1 X 5000 ml |
| GHK 10000 | 1 X 10000 ml |

**CLINICAL SIGNIFICANCE**

Glucose is the major carbohydrate present in the peripheral blood. The oxidation of glucose is the major source of cellular energy in the body. Glucose determinations are run primarily to aid in the diagnosis and treatment of diabetes mellitus. Elevated glucose levels may be associated with pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease, whereas low glucose levels may be associated with insulinoma, hypopituitarism, neoplasms, or insulin induced hypoglycemia.

**Method**

Enzymatic Method.

**PRINCIPLE**

Adenosine triphosphate promotes phosphorylation of glucose in a reaction catalyzed by hexokinase (HK), according to the following reaction:

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

Glucose-6-phosphate produced in the reaction above is oxidized to 6-phosphogluconate in the presence of nicotinamide adenine Dinucleotide (NAD), in reaction specifically catalyzed by glucose-6-Phosphate dehydrogenase (G-6-PDH). It yields one mole of NADH for Each mole of glucose-6-phosphate which is oxidized. The resulting Absorbance measured at 340 nm is directly proportional to the Concentration of glucose in the sample.

$$\text{Glucose-6-phosphate+NAD} \xrightarrow{\text{G-6-PDH}} \text{6-Phosphogluconate+NADH}$$

**REAGENT**

Reagent 1 : Buffer Reagent
Reagent 2 : Enzyme Reagent
Standard : 100 mg/dl.

**REAGENT PREPARATION**

Mix the reagent in the ratio of 4 part of R1 and 1 part of R2 and mix well.

**REAGENT STORAGE AND STABILITY**

When stored between 2-8°C the reagent is stable until the expiration date stated on the bottle and kit box label.

**WARNING AND PRECAUTIONS**

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.

**WASTE MANAGEMENT**

Please refer to local legal requirements.

**MATERIALS REQUIRED BUT NOT PROVIDED**

- NaCl solution 9 g/L
- General laboratory equipment

**SAMPLE COLLECTION AND PRESERVATION**

**Serum or heparin plasma or EDTA plasma**

Stability: 24 days at RT
7 days at 4 – 8°C
3 month at –20°C
Only freeze once! Discard contaminated specimens!

**ASSAY PROCEDURE**

**Operating Instructions**

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, Calibrator and samples to room temperature 18 - 28ºC, prior to analysis.

| AUTOMATED PARAMETERS | |
|---|---|
| Wavelength | 340 nm |
| Reaction Temperature | 37°C |
| Measurement | Against Reagent |
| Reaction | End point |
| Reaction Direction | Increasing |
| Sample Volume | 10 µl |
| Reagent Volume | 800 µl + 200 µl |
| Incubation | 5 min. |
| Linearity | 700 mg/dl |

**MANUAL ASSAY PROCEDURE**

**Pipette into Test Tubes**

| | Standard | Sample |
|---|---|---|
| Reagent 1 | 800 µl | 800 µl |
| Reagent 2 | 200 µl | 200 µl |
| Standard | 10 µl | - |
| sample | - | 10 µl |

Mix well and incubate at 37°C for 5 min. Measure the absorbance of sample and standard against reagent blank.

**SAMPLE DILUTIONS**

- This method is linear upto a concentration of 700 mg/dl.
- Dilute samples above this concentration 1:1 with 0.9% saline
- Repeat assay. Multiply the result by 2.

**CALCULATION**

Results are calculated, usually automatically by the instrument, as follows:

$$\text{Glucose (mg/dl)} = \frac{\text{Sample Abs.}}{\text{Standard Abs}} \times \text{Standard Concentration}$$

## CLIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-calibrator is recommended.

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.
Each laboratory should establish corrective action in case of deviations in control recovery

## PERFORMANCE CHARACTERISTICS

**WITHIN RUN**

| Sample | Mean Concentration | SD | CV % |
|---|---|---|---|
| Randox Level 2 | 112.60 | 1.28 | 1.14 |
| Randox Level 3 | 284.60 | 1.13 | 0.47 |

**RUN TO RUN**

| Sample | Mean Concentration | SD | CV % |
|---|---|---|---|
| Randox Level 2 | 112.05 | 0.90 | 0.81 |
| Randox Level 3 | 284.04 | 0.83 | 0.29 |

**LINEARITY**

The method is linear upto a concentration of 700 mg/dl. Dilute samples above this concentration 1:1 with 0.9% saline solution and repeat assay. Multiply the result by 2.

**REFERENCE VALUES**

| | |
|---|---|
| Serum/plasma | 74 – 106 mg/dl |

| | |
|---|---|
| Urine | <0.5 g/day, 1 – 15 mg/dl |

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

**LIMITATION OF THE PROCEDURE**

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

**INTERFERENCE**

- Hemoglobin: No interference found upto 500 mg/dL.
- Uric Acid: No interference found upto 20 mg /dL.
- Ascorbic Acid: No interference found upto 20 mg/dL.
- These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used.

**BIBLIOGRAPHY**

1. Bergmeyer HU. Methods of Enzimatic Analisys, 3ed, vol 6, Deerfield Beach: Verlag Chemie,1984;163-172
2. Bjorkem I, Blomstrand R, Falk O, Ohman G. Clin Chim Acta 1976;72:353-62
3. Bondar RJL, Mead D. Clin Chem 1974;20:586-90

**GLOSSARY OF SYMBOL**

| | |
|---|---|
|  | Consult Instruction for Use |
|  | For *in vitro* Diagnostic use only |
|  | Store between |
|  | Manufacturer |
|  | Keep away from sunlight |



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