TECO DIAGNOSTICS
1268 N. Lakeview Ave.
Anaheim, CA 92807
1-800-222-9880

GLUCOSE (HEXOKINASE) REAGENT SET

INTENDED USE
For the *in vitro* quantitative determination of glucose in human serum.

INTRODUCTION
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 are mainly associated with insulinemia or insulin-induced hypoglycemia.[1] A number of secondary factors also can contribute to elevated blood glucose levels. These include pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease.[2]

An enzymatic approach for glucose determination involves hexokinase coupled with glucose-6-phosphate dehydrogenase.[3] A revision of this approach is proposed by the U.S. Center For Disease Control as the reference method for glucose and forms the basis of the reagent for glucose.[4]

PRINCIPLE
Glucose + ATP $\xrightarrow{HK}$ G-6-P + ADP

G-6-P + NAD $\xrightarrow{G6PDH}$ 6-Phosphogluconate + NADH

The enzymatic hexokinase (HK) catalyzes the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6phosphate and adenosine diphosphate (ADP). In the presence of NAD, the enzyme glucose-6-phosphate dehydrogenase (G6PDH), oxidizes glucose-6-phosphate to 6-phosphogluconate. The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

REAGENT COMPOSITION
When reconstituted with distilled water as directed, the reagent contains the following:

Hexokinase 1,000 U/L; G6PDH 1,000 U/L; ATP 1.0 nm; NAD 1.0 mM; Buffer 100 mM pH = 7.5 $\pm$ 0.1 (30°C); nonreactive stabilizers and preservatives have been added.

PRECAUTIONS
Reagent is for "in vitro" diagnostic use only.

REAGENT PREPARATION
Reconstitute reagent vials with volume of distilled water stated on vial label. Swirl gently to dissolve.

REAGENT STORAGE
1. The dry reagent and standard should be stored refrigerated at 2-8°C.
2. Reconstituted reagent is stable for 48 hours at room temperature and for 30 days refrigerated at 2-8°C.

REAGENT DETERIORATION
Do not use if:
1. Reagent has an absorbance greater than 0.30 when measured against water at 340 nm.
2. The reagent fails to recover stated control values or meet stated linearity.
3. The reconstituted reagent develops turbidity, indicating contamination.

SPECIMEN COLLECTION
1. Either serum or plasma may be used.
2. Plasma or serum samples without preservatives should be separated from the cells or clot within a half hour of being drawn.
3. Glucose in separated unhemolyzed serum is generally stable up to eight hours at 25°C and up to 72 hours at 4°C.[5]
4. Glycolysis can be inhibited by collecting the specimen in sodium fluoride. Glucose in a sodium fluoride-oxalate mixture is reported to be stable up to 24 hours at 25°C.[5]

INTERFERING SUBSTANCES
Young et al. give a list of drug and other substances that may affect glucose values.[6]

MATERIALS PROVIDED
1. Glucose hexokinase reagent.
2. Glucose standard (100 mg/dl).

MATERIALS REQUIRED BUT NOT PROVIDED
1. Accurate pipetting devices.
2. Timer.
3. Test tubes and rack.
4. Spectrophotometer capable of reading at 340 nm.
5. Heating block or water bath (37°C).

PROCEDURE (AUTOMATED)
Refer to specific instrument application instructions.

PROCEDURE (MANUAL)
1. Appropriately label tubes: reagent blank, standard, control, sample, etc.
2. Pipette 1.0 ml of reagent into all tubes.
3. Add 0.005 ml (5 µl) of sample to respective tubes. Mix well. Incubate all tubes at 37°C for five (5) minutes.
4. After incubation, zero the spectrophotometer with the reagent blank at 340 nm.
5. Read and record the absorbance of all tubes.

* TC - MULTI PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.

Note: Alternate volume 3.0 ml reagent and 0.015 ml (15 µl) sample.

CALCULATIONS
Abs. = absorbance at 340 nm

$$\frac{\text{Sample Abs.}}{\text{Standard Abs.}} \times \text{Conc. of standard} = \text{Conc. of glucose (mg/dl)}$$

Example: Sample Abs. = 0.155
Standard Abs. = 0.164

$$\frac{0.155}{0.165} \times 100 = 93 \text{ mg/dl}$$

Note: To convert the results into SI units (mmol/L), multiply the result (mg/dl) by 0.0556.

LIMITATIONS
1. A "Sample Blank" should be prepared if the sample is moderately lipemic or icteric. This may be done by adding 0.005 ml (5 µl) of the sample to 1.0 ml of physiological saline (0.9% NaCl), mixing and taking absorbance reading at the assay wavelength against physiological saline. Subtract the "Sample Blank" absorbance from the "Sample" absorbance and calculate the concentration using the corrected absorbance.
2. Sample with values exceeding 600 mg/dl should be diluted 1:1 with saline, re-run and the final answer multiplied by two (2).
3. Extremely hemolyzed samples should not be used for the glucose assay.

QUALITY CONTROL
It is recommended that both normal and abnormal quality control sera be used routinely.

EXPECTED VALUES[5]
Normal range is reported to be 65-100 mg/dl. This range should serve only as a guideline. It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations. In a study of 50 samples, the expected values were found to be 65 - 112 mg/dl.

PERFORMANCE CHARACTERISTICS
1. Linearity: 600 mg/dl.
2. Comparison: Glucose (HK) was compared to a commercially available glucose hexokinase method with the resulting linear regression equation of Y = 1.10x + 0.33. Coefficient of correlation $R^2 = 0.99$. Twenty-nine patient sera and controls were assayed by two methods.
3. Precision:

Within Run

Mean (mg/dl)	S.D.	C.V.%
90.9	3.8	4.2
339.2	8.7	2.6

Run-to-Run

Mean (mg/dl)	S.D.	C.V.%
93.2	4.8	5.2
295.3	23.4	7.9

REFERENCES

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4. A Proposed Method for Determining Glucose Using Hexokinase and Glucose-6-phosphate Dehydrogenase, Public Health Service, Center for Disease Control, (1976).
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Manufactured by:

TECO DIAGNOSTICS
1268 N. LAKEVIEW AVE.
ANAHEIM, CA 92807
U.S.A.

CE EC REP
EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands