

Glucose Reagent

Hexokinase Method

PRODUCT SUMMARY

Stability	:	30 days at 2-8°C
Linear Range	:	Up to 42 mmol/L (756 mg/dL)
Specimen Type	:	Serum, plasma or urine
Method	:	Endpoint
Reagent Preparation	:	Add specified volume of distilled or deionised water.



SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Temperature Limitation
	For in vitro diagnostic use		Use by/Expiration Date
	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
	Catalogue number		Manufactured by
	Consult instructions for use		Xn - Harmful

INTENDED USE

This reagent is intended for the in vitro quantitative determination of glucose in human serum, plasma or urine.

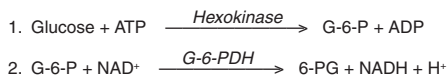
CLINICAL SIGNIFICANCE

The accurate estimation of glucose is important in the diagnosis and management of hyperglycaemia and hypoglycaemia. Hyperglycaemia may occur as a result of diabetes mellitus, in patients receiving glucose containing fluids intravenously, during severe stress and cerebrovascular accidents. Hypoglycaemia may be the result of an insulinoma, insulin administration, inborn errors of carbohydrate metabolism or fasting.¹ Often in the investigation of these disorders glucose determinations are performed in conjunction with various tolerance tests or stimulation tests. For a more detailed discussion of glucose metabolism the user should refer to a standard text book such as Kaplan.²

METHODOLOGY³

The Hexokinase / glucose-6-phosphate dehydrogenase method developed by the American Association of Clinical Chemistry and Centres for Disease Control has been accepted as the reference method for glucose determination. In this procedure protein free filtrates prepared by the Somogyi technique using ZnSO₄ / BaSO₄ precipitation are used. For routine laboratory use however serum or plasma without protein removal is the preferred method. The Glucose Hexokinase reagent is based on this reference method.

The series of reactions involved in the assay system is as follows:



- Hexokinase catalyses the phosphorylation of glucose by ATP producing ADP and glucose-6-phosphate.
- Glucose-6-phosphate is oxidised to 6-phosphogluconate with the reduction of NAD⁺ to NADH by G-6-PDH. The amount of NADH formed is proportional to the concentration of glucose in the sample and can be measured by the increase in absorbance at 340 nm.

Abbreviations

ATP	=	Adenosine-5'-triphosphate
ADP	=	Adenosine-5'-diphosphate
G-6-PDH	=	Glucose-6-phosphate dehydrogenase
G-6-P	=	Glucose-6-phosphate
6-PG	=	6-phosphogluconate
NAD ⁺	=	Nicotinamide Adenine Dinucleotide
NADH	=	Reduced NAD

REAGENT COMPOSITION

Active Ingredients	Concentration
Triethanolamine	20 mmol/L
ATP	1.65 mmol/L
NAD	1.06 mmol/L
Hexokinase (Recombinant Yeast)	>1,500 U/L
G-6-PDH (Recombinant Leuconostoc)	>1,500 U/L
pH 7.3 ± 0.1 at 20°C	

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains Sodium Azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Glucose Hexokinase Reagent Material Safety Data Sheet. **The Packaging of This Product Contains Dry Natural Rubber.** Exercise precaution when handling metal crimps and broken glass vials, as sharp edges can injure the user.

R22	Harmful if swallowed
S28	After contact with skin, wash immediately with plenty of soap and water.

REAGENT PREPARATION

Reconstitute the reagent with the volume of distilled or deionised water stated on the vial label.

STABILITY AND STORAGE

Prior to Use:

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

Reconstituted Reagent:

When stored capped at 2-8°C the reagent is stable for 30 days.

Indications of Reagent Deterioration:

- Turbidity;
- Reagent absorbance >0.2 at (340 nm, 1cm lightpath); and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING

Collection: The stability of glucose specimens is reduced by bacterial contamination and glycolysis. In order to inhibit glycolysis samples should be collected into tubes containing Sodium Fluoride. As soon as possible serum or plasma should be separated from the cells.

Serum: Use non-haemolysed serum.

Plasma: Use heparin.

Urine: If a delay in transport to the laboratory is expected the use of a chemical preservative such as merthiolate (0.23 mmol/L) is recommended.⁵

Storage: Serum or plasma glucose is stable for 4 hours at 30°C and 24 hours at 4°C. For long term storage samples should be placed in sealed containers and frozen at -10°C.^{4,5} Urine samples are stable for 1 day at 4°C.⁵

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm (334-365 nm).
- Distilled or deionised water for reagent preparation and related equipment, eg: pipettes.
- Analyser specific consumables, eg: sample cups.
- Normal and abnormal assayed control material.
- Calibrator or a suitable aqueous glucose standard.

ASSAY PROCEDURE

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	30/37°C
Primary Wavelength	340 nm (334 - 365nm)
Secondary Wavelength	380 nm (380 - 410nm)
Assay Type	End Point
Direction	Increase
Sample:Reagent ratio	1:100 - 1:150
e.g. Sample vol	3 µL
Reagent vol	300 µL
Incubation Time	180 seconds
Reagent Blank Limits	Low 0.0 AU
(340nm, 1cm lightpath)	High 0.2 AU
Linearity	Up to 42 mmol/L (756 mg/dL)
Sensitivity	0.017 ΔA per mmol/L
(340nm, 1cm lightpath)	(0.001 ΔA per mg/dL)

CALCULATIONS

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

$$\text{Glucose} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:

Absorbance of Calibrator	=	0.23
Absorbance of unknown	=	0.10
Value of Calibrator	=	13.1 mmol/L (236 mg/dL)

$$\text{Glucose} = \frac{0.10}{0.23} \times 13.1 = 5.7 \text{ mmol/L}$$

$$\text{Glucose} = \frac{0.10}{0.23} \times 236 = 103 \text{ mg/dL}$$

For urine specimens the results must be multiplied by the dilution factor and 24 hour collections by the volume in litres.

$$\text{Urine Glucose (mmol/24 hours)} = \text{Glucose Result (mmol/L)} \times \text{Dilution Factor} \times \text{Volume (L)}$$

Example:

Glucose result = 0.7 mmol/L (12.6 mg/dL)
 Dilution of Urine = Neat
 24 Hour volume of urine = 0.95 Litres

Urine Glucose = $0.7 \times 1 \times 0.95 = 0.67$ mmol/24 hours
 Urine Glucose = $12.6 \times 1 \times 0.95 = 11.97$ mg/24 hours

NOTES

- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- The test may be performed at 25°C or 30°C by increasing the incubation time to 10 minutes and 6 minutes respectively.
- May also be run at 334 or 365 nm
- Specimens with glucose values above 42 mmol/L (756 mg/dL) should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.
- Unit Conversion: mmol/L x 18 = mg/dL.

CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with and assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The Lot number of reagent changes
- Preventative maintenance is performed or a critical component is replaced
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values for this methodology should be run as unknown samples:-

- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.

Control results falling outside the established limits indicate the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control perform a calibration with fresh reagent, then repeat the test.
- If results remain out of control contact Technical Services or your local distributor

LIMITATIONS

- Studies to determine the level of interference from haemoglobin, bilirubin and lipaemia were carried out and the following results were obtained:

Haemoglobin: Avoid the use of haemolysed specimens.
Bilirubin: No interference from bilirubin up to 340 µmol/L (20 mg/dL).
Lipaemia: No interference from lipaemia, measured as triglycerides, up to 5.6 mmol/L (500 mg/dL).

- Young DS⁷ has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES

Serum/Plasma:⁹ 3.89 - 5.83 mmol/L (70 - 105 mg/dL)
 Urine:⁹ 0.28 - 0.83 mmol/L (5 - 15 mg/dL)

For the diagnosis of diabetes or impaired Glucose Tolerance (GT) the W.H.O. recommend the following criteria:¹⁰

	Plasma Venous	Capillary
Diabetes		
Fasting	≥7.8 mmol/L (≥140mg/dL)	≥7.8 mmol/L (≥140mg/dL)
2 hrs after glucose load	≥11.1 mmol/L (≥200mg/dL)	≥12.2 mmol/L (≥200mg/dL)
Impaired GT		
Fasting	<7.8 mmol/L (<140mg/dL)	<7.8 mmol/L (<140mg/dL)
2 hrs after glucose load	7.8-11.1 mmol/L (140-200 mg/dL)	8.9-12.2 mmol/L (160-220 mg/dL)

PERFORMANCE DATA

The following data was obtained with the Glucose Hexokinase Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on the specific analyser used.

IMPRECISION

Imprecision was evaluated using two levels of commercial control and following the NCCLS EP5-T procedure.¹¹

Within run:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.44 / 98	16.11 / 290
S.D. (mmol/L / mg/dL)	0.08 / 1.4	0.09 / 1.7
C.V. (%)	1.4	0.6

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.44 / 98	16.11 / 290
S.D. (mmol/L / mg/dL)	0.17 / 3.0	0.43 / 7.8
C.V. (%)	3.1	2.7

ACCURACY

Comparison studies were done using another commercially available glucose hexokinase reagent. Normal and abnormal patient serum and plasma samples were assayed in parallel. The results were compared by least squares regression and the following statistics were obtained.

Number of sample pairs	60
Range of sample results	1.9 - 26.1 mmol/L (34 - 469 mg/dL)
Mean of reference method results	5.4 mmol/L (97 mg/dL)
Mean of Glucose Hexokinase results	5.3 mmol/L (96 mg/dL)
Slope	0.97
Intercept	0.12 mmol/L (2.18 mg/dL)
Correlation coefficient	1.00

LINEARITY

When run as recommended the assay is linear up to 42 mmol/L (756 mg/dL).


SENSITIVITY

When run as recommended the sensitivity of the assay is 0.017ΔA per mmol/L or 0.001 ΔA per mg/dL (1cm light path, 340nm).

REFERENCES

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REF

Reorder Information

Catalogue No.	Configuration
1520-200A	20 x 10 mL
TR15015	20 x 20 mL
TR15003/1520-500	10 x 50 mL
TR15004	10 x 200 mL