Infinity™
Glucose Hexokinase Liquid Stable Reagent

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-8°C
Linear Range	:	0 - 45 mmol/L (0 - 810 mg/dL)
Specimen Type	:	Serum, plasma or urine
Method	:	Enzymatic Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		

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]

METHODOLOGY[3]
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 $ZnSO_4$ / $BaSO_4$ 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:

1. Glucose + ATP $\xrightarrow{Hexokinase}$ G-6-P + ADP

2. G-6-P + NAD^+ $\xrightarrow{G\text{-}6\text{-}PDH}$ 6-PG + NADH + H^+

1. Hexokinase catalyses the phosphorylation of glucose by ATP producing ADP and glucose-6-phosphate.
2. 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
Buffer	37.6 mmol/L
ATP	2.1 mmol/L
NAD	2.5 mmol/L
Hexokinase (Recombinant Yeast)	> 1500 U/L
G-6-PDH (Recombinant Leuconostoc)	> 2500 U/L
pH 7.7 ± 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 Infinity Glucose Hexokinase Liquid Stable Reagent Material Safety Data Sheet.

CAUTION: This product contains animal source material. Handle and dispose of this product as if it were potentially infectious.

REAGENT PREPARATION
The reagent is supplied ready to use.

STABILITY AND STORAGE
Prior to use:
When stored refrigerated at 2-8°C the reagent is stable until the expiry date stated on the bottle and kit box label.

Once opened:
Once opened, the reagent is stable until the expiry date stated on the bottle and kit box label when stored refrigerated at 2-8°C.
Indications of Reagent Deterioration:
- Turbidity;
- Reagent absorbance >0.5 (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.[4]
Storage: In separated, non-haemolysed serum or plasma, glucose is stable for up to 72 hours at 4°C or as long as 8 hours at 25°C.[2,5] In the presence of sodium fluoride, glucose is stabilized for as long as 3 days at room temperature.[6] For long term storage samples should be placed in sealed containers and frozen at -10°C.[7] Urine samples are stable for 1 day at 4°C.[4]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyzer capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm (334-365 nm).
- Analyzer specific consumables, e.g.: 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	37°C
Primary Wavelength	340 nm (334 - 365 nm)
Secondary Wavelength	380 nm (380 - 410 nm)
Assay Type	End Point
Direction	Increase
Sample:Reagent ratio	1:150
e.g. Sample vol	3 µL
Reagent vol	450 µL
Incubation Time	3 minutes
Reagent Blank Limits	Low 0.00 AU
(340 nm, 1cm lightpath)	High 0.50 AU
Linearity	0-45 mmol/L (0-810 mg/dL)
Analytical Sensitivity	0.038 ΔAbs per mmol/L
(340 nm, 1cm lightpath)	(0.002 ΔAbs 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 = 0.30
Absorbance of unknown = 0.10
Value of Calibrator = 13.2 mmol/L (238 mg/dL)

$$\text{Glucose} = \frac{0.10}{0.30} \times 13.2 = 4.4 \text{ mmol/L}$$

$$\text{Glucose} = \frac{0.10}{0.30} \times 238 = 79 \text{ mg/dL}$$

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

Urine Glucose (mmol/24 hours) = Glucose Result (mmol/L) x Dilution Factor x Volume (L)

Example:

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

Urine Glucose	=	0.7 x 1 x 0.95	=	0.67 mmol/24 hours
Urine Glucose	=	12.6 x 1 x 0.95	=	11.97 mg/24 hours

NOTES

1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. May also be run at 334 or 365 nm.
3. Specimens with glucose values above 45 mmol/L (810 mg/dL) should be diluted with isotonic saline and reassayed. Multiply results by the dilution factor.
4. Unit Conversion: mmol/L x 18 = mg/dL.

CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (e.g. 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 once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

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

1. Studies to determine the level of interference from haemoglobin, bilirubin (free and conjugated) and lipaemia were carried out. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 470 mg/dL.
   Free Bilirubin: No interference from free bilirubin up to 281 µmol/L (16.4 mg/dL).
   Conjugated Bilirubin: No interference from conjugated bilirubin up to 298 µmol/L (17.4 mg/dL).
   Lipaemia: No interference from lipaemia, measured as triglycerides, up to 23 mmol/L (2000 mg/dL).
2. Young DS[8] has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES

Fasting serum: [9]	4.11 - 5.56 mmol/L (74 - 100 mg/dL)
Urine:[9]	0.06 - 0.83 mmol/L (1 - 15 mg/dL)

For the diagnosis of diabetes, Impaired Fasting Glucose (IFG) or Impaired Glucose Tolerance (IGT) the W.H.O. recommend the following criteria:[10]

Diabetes

Fasting plasma glucose	≥7.0 mmol/L (≥126 mg/dL)
2 hrs after glucose load	≥11.1 mmol/L (≥200 mg/dL)

IFG

Fasting plasma glucose	6.1-6.9 mmol/L (110-125 mg/dL)

IGT

Fasting plasma glucose	≤7.0 mmol/L (≤126 mg/dL)
2 hrs after glucose load	7.8-11.0 mmol/L (140-199 mg/dL)

PERFORMANCE DATA

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

IMPRECISION

Imprecision was evaluated over a period of 20 days 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)	5.09 / 91.6	19.27 / 346.9
SD (mmol/L / mg/dL)	0.08 / 1.44	0.26 / 4.68
C.V. (%)	1.6	1.4

Total:	LEVEL I	LEVEL II
Number of data points	80	80
Mean (mmol/L / mg/dL)	5.09 / 91.6	19.27 / 346.9
SD (mmol/L / mg/dL)	0.20 / 3.6	0.85 / 15.3
C.V. (%)	3.9	4.4

METHOD COMPARISON

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

Serum/plasma:

Number of sample pairs	60
Range of sample results	2.3 - 26.7 mmol/L (41.4 - 480.6 mg/dL)
Mean of reference method results	6.25 mmol/L (112.5 mg/dL)
Mean of Infinity Glucose HK results	6.27 mmol/L (112.9 mg/dL)
Slope	1.021
Intercept	-0.13 mmol/L (-2.34 mg/dL)
Correlation coefficient	0.9993

Urine:

Number of sample pairs	60
Range of sample results	0.0 - 44.0 mmol/L (0.0 - 792.0 mg/dL)
Mean of reference method results	9.8 mmol/L (176 mg/dL)
Mean of infinity Glucose HK results	10.4 mmol/L (187 mg/dL)
Slope	1.086
Intercept	-0.29 mmol/L (-5.22 mg/dL)
Correlation coefficient	0.9962

LINEARITY

When run as recommended the assay is linear between 0 and 45 mmol/L (0 - 810 mg/dL).

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of the assay is 0.038ΔAbs per mmol/L or 0.002 ΔAbs per mg/dL (1cm light path, 340 nm).

REFERENCES

1. Zilva JF, Pannall PR. Carbohydrate Metabolism in "Clinical Chemistry in Diagnosis and Treatment". Lloyd-Luke London 1979, Chap 9: 174-214.
2. Kaplan LA, Pesce AJ (Ed) "Clinical Chemistry Theory, Analysis and Correlation". CV Mosby Company 1984. Chap 54: 1032-1035.
3. Farrance I. Clin Biochem Reviews 1987; 8: 55-68.
4. Shephard MDS, Mazzachi RD. The Clin Biochem 1983; 4: 61-7.
5. Tietz Textbook of Clinical Chemistry. (2nd Ed) Burtis and Ashwood. 1994; Chap 22: 959.
6. Chan et al; Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem., 35; 315-317, 1989.
7. Sherman S et al; Studies of the Stability of 18 Chemical constituents of Human Serum. Clin Chem. Vol 18, No. 12: 1498, 1972
8. Young DS, et al. Clin Chem 1975; 5: 1D-432D.
9. Tietz Textbook of Clinical Chemistry. (4th Ed) Burtis, Ashwood & Bruns 2005; VII: 2270-2271.
10. World Health Organisation Laboratory Diagnosis and Monitoring of Diabetes Mellitus. Reinauer H, et al. 2002; 16.
11. National Committee of Clinical Laboratory Standards. User evaluation of Precision Performance of Clinical Chemistry Devices NCCLS 1984; NCCLS publication EP5-T.

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REF

Reorder Information

Catalogue No.	Configuration
TR15421	2 x 125 mL