Glucose Hexokinase FS*

Order Information

Cat. No.	Kit size				
1 2511 99 10 021	R1	5 x 20 mL	+	R2	1 x 25 mL
1 2511 99 10 026	R1	5 x 80 mL	+	R2	1 x 100 mL
1 2511 99 10 023	R1	1 x 800 mL	+	R2	1 x 200 mL
1 2511 99 10 704	R1	8 x 50 mL	+	R2	8 x 12.5 mL
1 2511 99 10 917	R1	8 x 60 mL	+	R2	8 x 15 mL

Kits for use in conjunction with DiaSys CE applications.

Intended Use

Diagnostic reagent for quantitative in vitro determination of glucose in human serum, heparin plasma or urine on automated photometric systems.

Summary

Glucose is a monosaccharide and one of the most important carbohydrates for the human organism, as it is a metabolic substrate and a source of energy. The glucose concentration in blood is kept constant by several regulatory mechanisms. The main regulation occurs via secretion of insulin and glucagon. Primarily for the organism, the coverage of the steady glucose demand of the central nervous system with only minimal glucose reserves and the demand of erythrocytes is of major importance [1]. Glucose concentration in blood depends on nutritional status of an individual. Three conditions can be distinguished: Fasting status (8-10 h after the last nutritional intake), postprandial status (2-3 h after beginning of food intake) and postabsorptive status (6-12 h after beginning of food intake) [2]. Glucose measurement is recommended, whenever hypo- or hyperglycemia is suspected. Altered glucose can be the cause of many medical conditions. The main diseases causing elevated blood glucose levels are the different types of diabetes mellitus (DM). The primary purpose of glucose measurement is to diagnose DM respectively to define and monitor therapeutic interventions [2].

Method

Enzymatic UV test using hexokinase

Glucose is phosphorylated by hexokinase in the presence of ATP to form glucose-6-phosphate. Glucose-6-phosphate is converted in presence of NAD^+ by glucose-6-phosphate dehydrogenase to gluconate-6-phosphate and NADH + H^+. The increase of absorbance of NADH + H^+ is determined spectrophotometrically at a wavelength of 340 nm as endpoint measurement. The increase of absorbance is proportional to the glucose concentration in the sample.

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

$$\text{Glucose-6-phosphate + } NAD^+ \xrightarrow{\text{G6P-DH}} \text{Gluconate-6-phosphate + NADH + } H^+$$

Reagents

Components and Concentrations

R1:	TRIS buffer	pH 7.8	100 mmol/L
	Mg^{2+}		4 mmol/L
	ATP		2.1 mmol/L
	NAD		2.1 mmol/L
R2:	Mg^{2+}		4 mmol/L
	Hexokinase	(HK)	≥ 7.5 kU/L
	Glucose-6-phosphate dehydrogenase	(G6P-DH)	≥ 7.5 kU/L

Storage and Stability

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 – 8°C and contamination is avoided. Do not freeze and protect from light.

The open-vial stability of the reagent is 12 months until expiry date.

Warnings and Precautions

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 2 contains material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [3].
4. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
5. Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.
6. Please refer to the safety data sheets (SDS) and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only.

Waste Management

Refer to local legal requirements for chemical disposal regulations as stated in the relevant SDS to determine the safe disposal.

Warning: Handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Reagent Preparation

The reagents are ready to use.

Materials Required

General laboratory equipment

Specimen

Human serum, heparin plasma or urine

Only use suitable tubes or collection containers for specimen collection and preparation.

When using primary tubes, follow the manufacturer's instructions.

Separate at the latest 1h after blood collection from cellular contents.

Stability in serum/plasma after addition of a glycolytic inhibitor (fluoride, monoiodacetate, mannose) [4]:

2 days	at	20 – 25°C
7 days	at	4 – 8°C
1 day	at	-20°C

Only freeze once. Discard contaminated specimens.

Stability in serum (separated from cellular contents, hemolysis free) without adding a glycolytic inhibitor [5,6]:

8 h	at	25°C
72 h	at	4°C

Discard contaminated specimens.

Stability in urine [4]:

2 h	at	20 – 25°C
2 h	at	4 – 8°C
2 days	at	-20°C

Only freeze once. Discard contaminated specimens.

Assay Procedure

Basic settings for BioMajesty® JCA-BM6010/C

Wavelength	340/410 nm
Temperature	37°C
Measurement	Endpoint
Sample/Calibrator	1.0 µL
Reagent 1	80 µL
Reagent 2	20 µL
Addition reagent 2	Cycle 19 (286 s)
Absorbance 1	Cycle 17/18 (231 s/244 s)
Absorbance 2	Cycle 41/42 (586 s/600 s)
Calibration	Linear

Calculation

With Calibrator

$$\text{Glucose [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal.}} \times \text{Conc. Cal. [mg/dL]}$$

Conversion Factor

Glucose [mg/dL] x 0.05551 = Glucose [mmol/L]

Calibrators and Controls

DiaSys TruCal U is recommended for calibration. Calibrator values have been made traceable to the reference method gas chromatography – isotope dilution mass spectrometry (GC-IDMS). Glucose Standard FS may be used alternatively for calibration. Use DiaSys TruLab N and P or TruLab Urine Level 1 and Level 2 for internal quality control. All target values of the controls are traceable to DiaSys reagent/calibrator system. Quality control must be performed after calibration. Control intervals and limits have to be adapted to the individual requirements of each laboratory. Results must be within the defined ranges. Follow the relevant legal requirements and guidelines. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL
TruLab Urine Level 1	5 9170 99 10 062	20 x 5 mL
	5 9170 99 10 061	6 x 5 mL
TruLab Urine Level 2	5 9180 99 10 062	20 x 5 mL
	5 9180 99 10 061	6 x 5 mL
Glucose Standard FS	1 2500 99 10 030	6 x 3 mL

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

Serum/Plasma

Measuring range up to 500 mg/dL, linearity is given within ± 5%. When values exceed this range, samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result multiplied by 3.

Limit of detection**	1 mg/dL

Interference by	Interferences ≤ 10% up to	Analyte concentration [mg/dL]
Ascorbic acid	30 mg/dL	70.0
Bilirubin (conjugated)	60 mg/dL	69.5
Bilirubin (unconjugated)	60 mg/dL	69.9
Hemolysis	500 mg/dL	70.1
Lipemia (triglycerides)	2000 mg/dL	70.3

For further information on interfering substances, refer to the literature [7-9].

Precision			
Repeatability (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	77.5	91.9	267
CV [%]	0.732	0.865	0.566
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	57.2	111	332
CV [%]	1.42	1.50	1.13

Method comparison (n=100)	
Test x	Competitor Glucose Hexokinase (BioMajesty® JCA-BM6010/C)
Test y	DiaSys Glucose Hexokinase FS (BioMajesty® JCA-BM6010/C)
Slope	1.01
Intercept	0.859 mg/dL
Coefficient of correlation	0.999

Urine

Measuring range up to 500 mg/dL, linearity is given within ± 5%. When values exceed this range, samples should be diluted 1 + 10 with NaCl solution (9 g/L) and the result multiplied by 11.

Limit of detection**	1 mg/dL

Precision			
Repeatability (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	27.9	98.9	274
CV [%]	0.823	1.31	0.823
Between day (n=20)	**Sample 1**	**Sample 2**	**Sample 3**
Mean [mg/dL]	27.9	99.4	272
CV [%]	1.81	1.62	0.989

Method comparison (n=100)	
Test x	Competitor Glukose Hexokinase (BioMajesty® JCA-BM6010/C)
Test y	DiaSys Glucose Hekokinase FS (BioMajesty® JCA-BM6010/C)
Slope	0.985
Intercept	-0.380 mg/dL
Coefficient of correlation	0.999

** lowest measurable concentration which can be distinguished from zero; mean + 3 SD (n = 20) of an analyte free specimen.

Reference Range [2]

	[mg/dL]	[mmol/L]
Newborns		
Cord blood	63 – 158	3.5 – 8.8
1 h	36 – 99	2.0 – 5.5
2 h	39 – 89	2.2 – 4.9
5 – 14 h	34 – 77	1.9 – 4.3
20 – 28 h	46 – 81	2.6 – 4.5
44 – 52 h	48 – 79	2.7 – 4.4
Children (fasting)	60 – 99	3.3 – 5.5
Adults (fasting)		
Serum/Plasma	60 – 95	3.3 – 5.3
Urine	≤ 16.5	≤ 0.91

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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