



GLUCOSE - L



Enzymatic colorimetric method for the quantitative determination of Glucose in serum, plasma and urine

ORDER INFORMATION

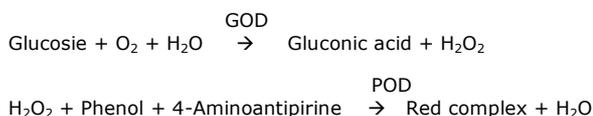
REF	Kit size
GA4575 00	12x50 ml
GD0574 00	4x250 ml
KL4575 00	10x60 ml
BK4575 00	2x60 ml

INDICATION

Glucose determination is used for the diagnosis and monitoring of diabetes and others disorders of glucidic metabolism.

METHOD PRINCIPLE

Glucose is transformed by glucose oxidase (GOD) in gluconic acid and hydrogen peroxide, which, in presence of peroxidase (POD), reacts with phenol and 4-aminoantipirine to form a red complex, whose intensity at 505 nm is proportional to the glucose concentration in the sample.



COMPOSITION

REAGENT A:

Phosphate buffer pH 7.4	25 g/l
Phenol	< 0.9 g/l
4-Aminoantipirine	0.4 mmol/l
GOD	≥ 30 kU/l
POD	≥ 1 kU/l
NaN ₃	0.95 g/l

STANDARD:

	1x5 ml
D-Glucose	100 mg/dl (5.55 mmol/l)
Benzoic acid	< 14.7 mmol/l

Verified against NIST reference material.

Preparation

Reagents are liquids ready to use.

Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light.

The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, plasma (EDTA, monoiodoacetate), urine 24h.

Stability:

	Temperature	
	15-25 °C	4-8 °C
Serum/plasma (after adding glycolytic inhibitors as NaF, KF):	1 day	7 days
Urine (put in the container 5 ml of glacial acetic acid):	2 hours	2 hours

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use controls with known glucose concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes :

	Blank	Standard	Sample
Reagent A	1000 µl	1000 µl	1000 µl
Distilled H ₂ O	10 µl		
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix and incubate for **10 minutes at 37 °C**.

Read the absorbance (A) of the standard and samples at **505 (590-530) nm** against Blank.

Colour is stable for 60 minutes, protected from light.

Note:

- Reaction volumes can be proportionally changed.
- For lipemic or icteric samples, utilize a sample blank (0.01 ml sample and 1.0 ml of physiological solution).
- For plasma or serum concentration > of 500 mg/dl dilute sample 1:2 with NaCl (9 g/l) solution and multiply the result by 2.
- For urine concentration > of 500 mg/dl dilute sample 1:10 with distilled water (9 g/l) and multiply the result by 10.

CALCULATION OF RESULTS

Serum, plasma:

$$\text{Glucose, mg/dl} = \frac{A \text{ sample}}{A \text{ standard}} \times 100$$

Urine:

$$\text{Glucose, g/24h} = \frac{A \text{ sample}}{A \text{ standard}} \times 1/24\text{h}$$

Conversion factor

$$\text{Glucose [mg/dl]} \times 0.05551 = \text{Glucose [mmol/l]}$$

REFERENCE VALUES

Serum/Plasma

Adults:	70÷115 mg/dl (3.9÷6.4 mmol/l)
Newborn:	20÷80 mg/dl (3.9÷6.4 mmol/l)
Children < 5 years:	values 10-15% lower of adults ones

Urine

Absent in urine of healthy subjects on fasting.

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three controls at different glucose concentration. The obtained results are reported in the following table:

Sample	Mean (mg/dl)	Within-run		Between-run	
		SD	%CV	SD	%CV
Serum 1	39.5	0.71	1.8	1.25	3.2
Serum 2	91.7	2.06	2.2	2.83	3.1
Serum 3	247.9	5.95	2.4	7.09	2.9

Linearity

The assay is linear up to 500 mg/dl (28 mmol/l).

Sensitivity

Test sensitivity, in terms of limit of detection, is 1 mg/dl (0.05 mmol/l).

Correlation

A correlation study comparing the present method with a commercial one gave the following results:

$$y = 1.0234x - 7.0672 \text{ mg/dl} \quad r = 0.9964$$

Interferences

Bilirubin > 20 mg/dl
Hemoglobin > 400 mg/dl
Triglycerides > 250 mg/dl

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes. The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

1. Trinder P: Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Bioch*, 6:24 (1969).
2. Chitto G, Fabi A, Franzini C, Galletta G, Leonardi A, Marelli M, Morelli AM: Variabilità biologica intra-individuo: rassegna della letteratura, contributo sperimentale e considerazioni critiche. *Biochimica Clinica*, 18, 10:673 (1994).
3. Kaplan LA, Pesce AJ: "Clinical Chemistry", Mosby Ed. (1996).
4. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
5. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.