CATACHEM

GLUCOSE REAGENT KITS
C124-12, C124-06, C124-07

Contents	Product No.	Package
Glucose Reagent	C124-12	6 x 13 mL
Glucose Reagent	C124-06	6 x 25 mL
Glucose Reagent	C124-07	6 x 50 mL

REAGENT PREPARATION

This reagent is packaged ready for use.
No preparation is required.

REAGENT STORAGE AND STABILITY

Store unopened reagent at 2-8°C.
When stored as directed, the reagent is stable until the expiration dated stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
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CATACHEM

GLUCOSE REAGENT KITS C124-12, C124-06, C124-07 MANUAL/AUTOMATED PROCEDURE

Intended Use
For **IN VITRO quantitative** determination of Glucose in serum.

Clinical Significance
Measurements of Glucose are primarily used for diagnosing diabetes, nephritis, pancreatic disease, hyperthyroidism, hypopituitarism, hyperinsulinism, cretinism, and myxedema, as well as for monitoring the causes and treatments. (1)

Method History
The specificity of the hexokinase (HK) (EC 2.7.11) and the glucose-6-phosphate dehydrogenase (G-6-PDH) (EC 1.1.1.49) enzyme reactions for the determination of serum Glucose was first reported by Slein in 1950. (2) The Food and Drug Administration has proposed as the reference method for measurement of Glucose, a totally enzymatic procedure using HK and G6PDH. (3) Catachem Glucose Hexokinase method is based upon the work of L.P. Leon, et al.

Method Principle
The enzyme hexokinase catalyzes the phosphorylation of Glucose in the presence of ATP and magnesium ions. The resultant glucose-6-phosphate is then oxidized to 6-phosphoglucono lactone with concomitant reduction of nicotinamide adenine dinucleotide (NAD). The amount of NADH produced is proportional to the Glucose present in the serum sample and it is quantified at 340nm. The reaction scheme illustrates the reactions that occur in this method.

$$\text{D-Glucose} + (\text{ATP}) \xrightarrow{\text{HK}} \text{D-gluc-6-phosphate} + (\text{ADP}) + H^+$$

$$\text{D-Glucose-6-phos} + \text{NAD} \xrightarrow{\text{G6PDH}} \text{6-phosphoglucono-lactone}$$

Reagent Content
Active ingredients in the reagents will be approximately as follows:

Hexokinase Reagent
Each vial contains:

Buffer	
Hexokinase	≥ 800 U/L
G-6-PDH	≥ 800 U/L
Adenosine-5' - triphosphate	0.83 mM
Nicotinamide-Adenine Dinucleotide	1.21 mM
Non-reactive ingredients and stabilizers	

Precautions
Avoid contact with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagent
Catachem Glucose Reagent is packaged ready-for-use. No preparation is required.

Reagent Storage And Stability
Store the unopened reagent at 2-8°C. When stored as directed, this reagent is stable until the expiration date stated on the label. Upon opening and when stored capped when not in active use, the Working Glucose Reagent is stable for 60 days at 2-8°C. The Catachem Glucose Hexokinase Reagents have been tested to reflect shipping conditions and are stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Specimen Collection And Preparation
Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In serum the glucose concentration is stable for eight hours at 25˚C and 72 hours at 4˚C. (1)

Quality Control
To ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem's Catacal (C1200-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

Interfering Substances
A comprehensive discussion has been reported on the effect of interfering substances on various Glucose methods including the Hexokinase/G6PDH method. (4) A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et. al. (5)

Expected Values
The normal range for human samples for this assay as performed below is 70 mg/dL to 105 mg/dL for individuals less than 50 years of age and 85 mg/dL to 125 mg/dL for individuals over 50 years of age.

These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area in which the laboratory is located.

Analytical Parameters

Wavelength	340 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	endpoint
Reaction Time	5 min
Reagent Volume	1.0 ml

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Sample Volume	0.01 ml (10uL)
Total Volume	1.01 ml
Sample-to-reagent Ratio	1:101

Assay Procedure

1. Pipette 1.0 ml of Glucose Reagent into each of three cuvettes marked “calibrator”, sample” and “blank”.
2. Pipette 0.01 ml (10 uL) of calibrator or sample into their respective cuvettes. Mix all cuvettes well.
3. Incubate all cuvettes for 5 minutes at 37˚C.
4. Set spectrophotometer wavelength at 340 nm and zero the instrument with the cuvette marked “blank”.
5. Read the “calibrator” and “sample” absorbencies.
6. Calculate the glucose concentration (mg/dL) in the sample(s), as shown in results and calculations.

Results And Calculations

$$\text{Gluc (mg/dL)} = \frac{\text{sample abs}}{\text{calibrator abs}} \times \text{calibrator (mg/dL)}$$

Example:

Sample absorbance = 0.300

Calibrator absorbance = 0.250

Calibrator (mg/dL) = 200

$$\text{Glucose (mg/dL)} = \frac{0.300}{0.250} \times 200 = 240 \text{ mg/dL}$$

Method Performance Characteristics

***Sensitivity*:** 0.0008 - 0.0012 absorbance units per mg/dL.
***Linear Range*:** 0-600 mg/dL.
***Precision*:** Within-run and day-to-day precision is summarized as follows:

Precision Study

Glucose	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
58	1.30	2.40	3.10	5.20
289	1.80	0.70	7.40	2.50
475	3.50	0.70	5.00	1.00

Correlation

A comparison of this method using an automated analyzer and a reference method based upon the Hexokinase/G6PDH reaction resulted in the following regression statistics.

Range	=	60 - 472 mg/dL
N	=	160
Y	=	0.993 x +2.0
r	=	0.999
Sy.x	=	2.9

References

1. Fundamentals of Clinical Chemistry. Edited by Norbert W. Tietz.WB Saunders Company, Philadelphia.
2. Slein MW, Cori GT, Cor, CF. J Biol Chem 186:763-780 (1950).
3. United States Department of Health, Education and Welfare, Food and DrugAdministration. In Vitro Diagnostic Products for Human Use, Proposed Establishment of Product Class Standard for Detection of Measurement of Glucose. Fed Regist. 39,126, 24136-24147 (1974).
4. Leon LP, Sansur M, Snyder RL and Horvath C. Clin Chem 23, No. 9 (1977).
5. Young DS, Pestaner LD, Gibberman V. Clin Chem 21, No 5 (1975).

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