Glucose Colorimetric Assay Kit

Item No. 10009582

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size
10010098	Glucose Assay Standard	1 vial/300 µl
700003	Sodium Phosphate Assay Buffer	1 vial/10 ml
10010100	Glucose Colorimetric Enzyme Mixture	4 vials
400014	96-Well Solid Plate (Colorimetric Assay)	2 plates
400012	96-Well Cover Sheets	2 covers

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3640

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure absorbance between 500-520 nm
2. Adjustable pipettes and a multichannel or repeating pipette
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Glucose, a monosaccharide (or simple sugar), is the most important carbohydrate in biology. Transported *via* the blood stream, it is the primary source of energy for the body's cells. Glucose levels are tightly regulated in the human body. Failure to maintain blood glucose in the normal range leads to conditions of persistently high (hyperglycemia) or low (hypoglycemia) blood sugar. Diabetes mellitus, characterized by persistent hyperglycemia, is the most prominent disease related to improper blood sugar regulation.

The determination of glucose levels in blood is critical in the control of diabetes. A dinitrosalicylic acid (DNS) assay has been available since 1955 but more recently, several enzymatic assays using either hexokinase-glucose-6-phosphate dehydrogenase or glucose oxidase-peroxidase for glucose quantification have been developed.[1-3] The nonenzymatic assay quantitates all reducing sugars whereas the enzymatic assay is specific for glucose, allowing for more accurate quantification.

About This Assay

Cayman's Glucose Colorimetric Assay Kit provides a simple, reproducible, and sensitive tool for assaying glucose in plasma, serum, and urine. In this assay, glucose is oxidized to δ-gluconolactone with concomitant reduction of the flavin adenine dinucleotide (FAD)-dependent enzyme glucose oxidase (see Figure 1 on page 7; reaction 1). The reduced form of glucose oxidase is regenerated to its oxidized form by molecular oxygen to produce hydrogen peroxide (reaction 2). Finally, with horseradish peroxidase as a catalyst, hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminoantipyrine (also called 4-aminophenazone) to generate a pink dye with an optimal absorption at 514 nm (reaction 3).[4]

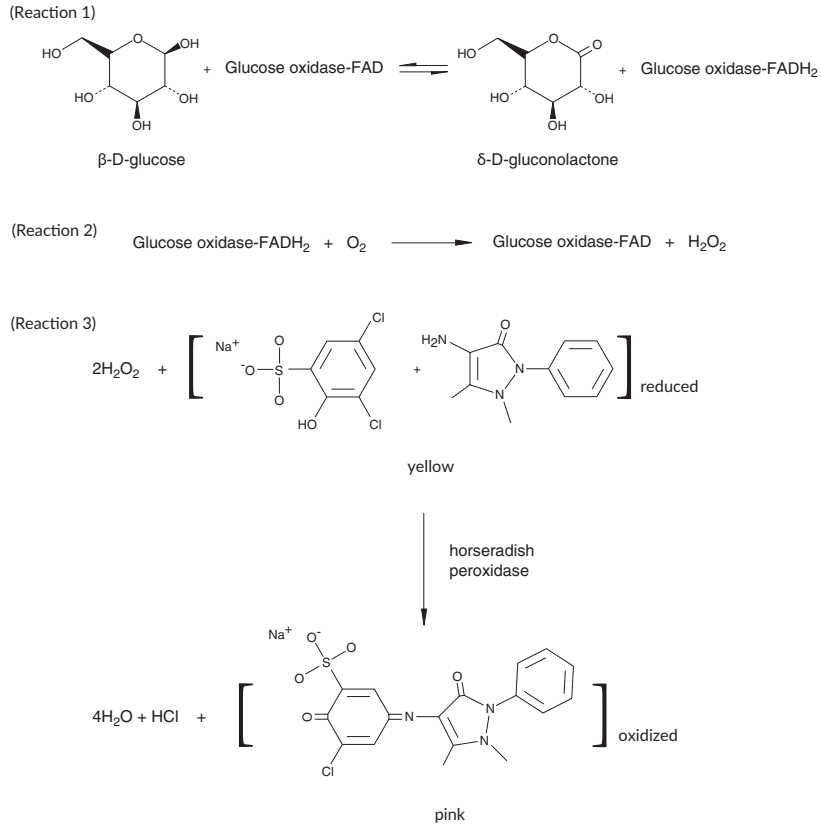


Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Glucose Assay Standard - (Item No. 10010098)**

   This vial contains 300 µl of 1,000 mg/dl glucose. It is ready to use as supplied to prepare the standard curve. Sufficient Standard is provided to prepare four standard curves.

2. **Sodium Phosphate Assay Buffer - (Item No. 700003)**

   This vial contains 10 ml of 250 mM sodium phosphate, pH 7.2. Mix the contents of the vial with 40 ml of HPLC-grade water. This solution is used to prepare the Glucose Standards and for dilution of the Enzyme Mixture. The diluted Buffer is stable for three months at 4°C.

3. **Glucose Colorimetric Enzyme Mixture - (Item No. 10010100)**

   This vial contains a lyophilized enzyme mixture. Reconstitute 1 vial with 6 ml of diluted Assay Buffer and mix well. This reconstituted solution is now ready to use in the assay. The reconstituted solution is stable for at least one hour when stored at 4°C. One vial of the Enzyme Mixture is sufficient to assay 60 wells.

Sample Preparation

Plasma

Typically, normal human plasma has glucose concentrations in the range of 70-110 mg/dl.[5]

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month when stored at -80°C.
3. Dilute plasma 1:5 with diluted Assay Buffer before assaying.

Serum

Typically, normal human serum has glucose concentrations in the range of 70-110 mg/dl.[5]

1. Collect blood without using an anticoagulant.
2. Allow blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month when stored at -80°C.
4. Dilute serum 1:5 with diluted Assay Buffer before assaying.

Urine

Typically, normal human urine has glucose concentrations in the range of 1-15 mg/dl.[5]

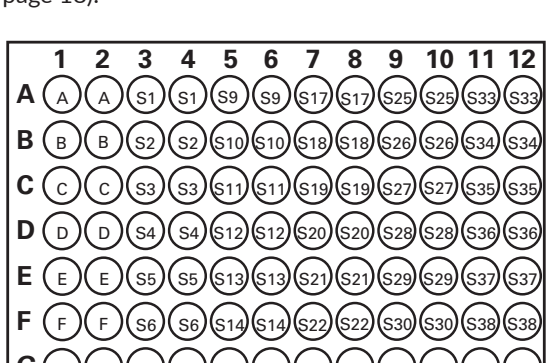
1. Urine does not require any special treatments. If not assaying the same day, freeze at -80°C.

NOTE: Glucose values from urine samples can be standardized using Cayman's Creatinine (urinary) Assay Kit (Item No. 500701).

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of glucose standards and samples to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 18).



A-H = Standards
S1-S40 = Sample wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all wells.
- The incubation temperature is 37°C.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the standards and samples be assayed at least in duplicate.
- Monitor the absorbance at 500-520 nm using a plate reader.

Standard Preparation

Mix 50 µl of the 1,000 mg/dl Glucose Standard (Item No. 10010098) with 450 µl of diluted Assay Buffer to make a 100 mg/dl stock. Label eight clean glass test tubes or polystyrene tubes A-H. Add the amount of Glucose Standard and Assay Buffer to each tube as described in Table 1. The diluted Glucose Standards are stable for two hours at room temperature.

Tube	Glucose Stock (µl) (100 mg/dl)	Assay Buffer (µl)	Glucose Concentration (mg/dl)
A	0	200	0
B	5	195	2.5
C	10	190	5
D	15	185	7.5
E	20	180	10
F	30	170	15
G	40	160	20
H	50	150	25

Table 1. Preparation of Glucose Standards.

Performing the Assay

1. **Glucose Standard wells -** Add 85 µl of diluted Assay Buffer and 15 µl of each Standard (tubes A-H) to two wells (see suggested plate configuration, Figure 2, page 10).
2. **Sample wells -** Add 85 µl of diluted Assay Buffer and 15 µl of sample to two wells.
3. Initiate the reaction by adding 100 µl of Enzyme Mixture to all standard and sample wells.
4. Cover the plate with the plate cover and incubate for 10 minutes at 37°C.
5. Remove the plate cover and read the absorbance at 500-520 nm using a plate reader.

ANALYSIS

Calculations

1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of the standard A (0 mg/dl) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Plot the corrected absorbance values (from step 2 above) of each standard as a function of the concentration of glucose (see Table 1, page 12).
4. Calculate the concentration of glucose for each sample from the standard curve. An example of the glucose standard curve is shown in Figure 3, see page 15.

$$\text{Glucose (mg/dL)} = \left[\frac{\text{(Corrected absorbance) - (y-intercept)}}{\text{Slope}}\right] \times \text{dilution}$$

Performance Characteristics

Sensitivity:

When a series of thirty-six human serum and urine samples were assayed on the same day, the intra-assay coefficient of variation was 4.6% and 8.1%, respectively. When a series of thirty-six human serum and urine samples were assayed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 1.7% and 11.3%, respectively. The lower limit of detection (LLOD) for this assay is approximately 0.23 mg/dl.

Representative Glucose Standard Curve

The standard curve presented here is an example of the data typically produced with this kit. Your results may vary, and therefore should not be directly compared to these samples. You must run a new standard curve.

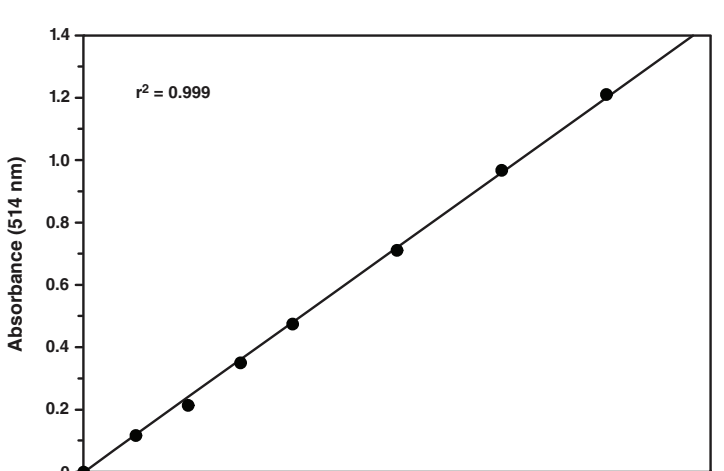


Figure 3. Glucose standard curve

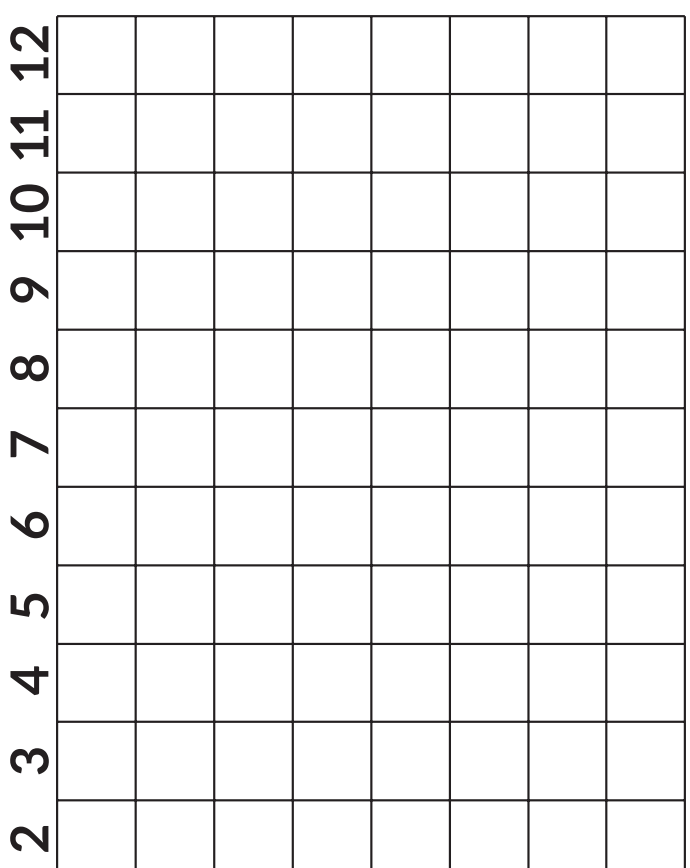
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No glucose was detected in the sample and standard wells	Enzyme Mixture was not prepared or stored correctly	Prepare fresh Enzyme Mixture and re-assay
Sample absorbance values are above highest point in standard curve	Glucose concentration was too high in the samples	Dilute samples with assay buffer and re-assay; *NOTE: Remember to account for the dilution factor when calculating glucose concentration*

References

1. Bernfeld, P. In amylase, α and β, Chapter 17, *in* Methods in Enzymology. Colowick, S.P. and Kaplan, N.O., editors, 1, Academic Press, New York, 149-158 (1955).
2. Carroll, J. A colorimetric serum glucose determination using hexokinase and glucose-6-phosphate dehydrogenase. *Biochem. Med.* **4**, 171-180 (1970).
3. Sugiura, M. and Hirano, K. A new colorimetric method for determination of serum glucose. *Clin. Chim. Acta* **75**, 387-391 (1977).
4. Frost, L.D. Glucose assays revisited: Experimental determination of the glucose concentration in honey. *Chemical Educator* **9(4)**, 239-241 (2004).
5. Slein, M.W. and Bergmeyer, H.U. Methods of Enzymatic Analysis 117-123 (1963).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.