Glucose Colorimetric Assay Kit

Item No. 10009582

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**TABLE OF CONTENTS**

**GENERAL INFORMATION**
- 3 Materials Supplied
- 4 Safety Data
- 4 Precautions
- 4 If You Have Problems
- 5 Storage and Stability
- 5 Materials Needed but Not Supplied

**INTRODUCTION**
- 6 Background
- 6 About This Assay

**PRE-ASSAY PREPARATION**
- 8 Reagent Preparation
- 9 Sample Preparation

**ASSAY PROTOCOL**
- 10 Plate Setup
- 12 Standard Preparation
- 13 Performing the Assay

**ANALYSIS**
- 14 Calculations
- 14 Performance Characteristics

**RESOURCES**
- 16 Troubleshooting
- 17 References
- 18 Plate Template
- 19 Notes
- 19 Warranty and Limitation of Remedy

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**GENERAL INFORMATION**

**Materials Supplied**

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

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: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST
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 \( \delta \)-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-aminophenazone (also called 4-aminoantipyrine) to generate a pink dye with an optimal absorption at 514 nm (reaction 3).4

![Reaction 1](image1)

\[
\begin{align*}
\beta-D-glucose + Glucose oxidase-FAD & \rightarrow \delta-D-gluconolactone + Glucose oxidase-FADH_2 \\
\text{(Reaction 1)}
\end{align*}
\]

![Reaction 2](image2)

\[
\begin{align*}
\text{Glucose oxidase-FADH}_2 + O_2 & \rightarrow \text{Glucose oxidase-FAD} + H_2O_2 \\
\text{(Reaction 2)}
\end{align*}
\]

![Reaction 3](image3)

\[
\begin{align*}
2H_2O_2 + \left[ \begin{array}{c}
\text{Na}^+ \\
\text{H_2N} \\
\text{O} \\
\text{S} \\
\text{O-Na}^+ \\
\end{array} \right] & \rightarrow \left[ \begin{array}{c}
\text{N} \\
\text{N} \\
\text{O} \\
\text{H_2N} \\
\text{Cl} \\
\text{O} \\
\text{S} \\
\text{O-Na}^+ \\
\end{array} \right] \text{reduced} \rightarrow \left[ \begin{array}{c}
\text{N} \\
\text{N} \\
\text{O} \\
\text{H_2N} \\
\text{Cl} \\
\text{O} \\
\text{S} \\
\text{O-Na}^+ \\
\end{array} \right] \text{oxidized} \\
4H_2O + HCl & \rightarrow \left[ \begin{array}{c}
\text{N} \\
\text{N} \\
\text{O} \\
\text{H_2N} \\
\text{Cl} \\
\text{O} \\
\text{S} \\
\text{O-Na}^+ \\
\end{array} \right] \text{pink} \\
\text{(Reaction 3)}
\end{align*}
\]

Figure 1. Assay scheme
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).

<p>| | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
<td>G</td>
<td>H</td>
<td></td>
<td></td>
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<td>S6</td>
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<td>S15</td>
<td>S16</td>
<td>S17</td>
<td>S18</td>
<td>S19</td>
<td>S20</td>
</tr>
<tr>
<td>C</td>
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<td>S23</td>
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<td>S26</td>
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<tr>
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<td>D</td>
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<td>S76</td>
<td>S77</td>
<td>S78</td>
<td>S79</td>
<td>S80</td>
</tr>
</tbody>
</table>

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.

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

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.
**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.

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

**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.

![Representative Glucose Standard Curve](image-url)
# Troubleshooting

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

## References

Warranty and Limitation of Remedy

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