Glucose Colorimetric Assay Kit
Item No. 10009582

Customer Service 800.364.9897 * Technical Support 888.526.5351
www.caymanchem.com
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GENERAL INFORMATION

Materials Supplied

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item Description</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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.
Precautions

Please read these instructions carefully before beginning this assay. For research use only. Not for human or diagnostic use.

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 bloodstream, 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. 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. The glucose assay uses the glucose oxidase-peroxide reaction for the determination of glucose concentrations. 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 6; equation 1). The reduced form of glucose oxidase is regenerated to its oxidized form by molecular oxygen to produce hydrogen peroxide (equation 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 (equation 3).
**Pre-Assay Preparation**

**Reagent Preparation**

1. **Glucose Assay Standard - (Item No. 10010098)**
   The 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)**
   The vial contains 10 ml of 250 mM sodium phosphate, pH 7.2. Dilute the contents of the vial with 40 ml of HPLC-grade water. This solution is used to prepare the diluted Glucose Standards and for the dilution of the Enzyme Mixture. The diluted Buffer is stable for three months at 4°C.

3. **Glucose Colorimetric Enzyme Mixture - (Item No. 10010100)**
   The 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 evaluate 60 wells.

![Assay Scheme](Figure 1)
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 while 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 while 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).

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, below. We suggest you record the contents of each well on the template sheet provided (see page 19).

A-H = Standards
S1-S40 = Sample Wells

**Figure 2. Sample plate format**
Pipetting Hints

- It is recommended that an adjustable 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

Dilute 50 µl of the 1,000 mg/dl Glucose Standard with 450 µl of diluted Assay Buffer to make a 100 mg/dl stock. Take eight clean 12 x 75 mm glass test tubes or polystyrene tubes and label them 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. Glucose standards to be assayed along with samples.
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 9).

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 11).

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 14.

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

Performance Characteristics

**Precision:**

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.

**Assay Range:**

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 0-25 mg/dl.
Representative Glucose Standard Curve

The standard curve presented here is an example of the data typically provided with this kit; however, your results will not be identical to these. You must run a new standard curve - do not use these to determine the values of your samples.

![Glucose standard curve graph]

Figure 3. Glucose standard curve

### Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
</table>
| 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 correctly or following reconstitution had exceeded the one hour stability limit | Prepare a fresh Enzyme Mixture and re-assay      |
| Sample absorbance values are above highest point in standard curve | Glucose concentration was too high in the sample | Dilute samples with assay buffer and re-assay; NOTE: Remember to account for the dilution factor when calculating glucose concentration |
References


Related Products

- Acylated Ghrelin (human) Express EIA Kit - Item No. 10006306
- Acylated Ghrelin (mouse, rat) Express EIA Kit - Item No. 10006307
- Adipogenesis Assay Kit - Item No. 10006908
- Adiponectin (human) EIA Kit - Item No. 500641
- AgRP (human) EIA Kit - Item No. 10007615
- ANGPTL3 (human) EIA Kit - Item No. 580170
- Calcium Assay Kit - Item No. 700550
- Chloride Colorimetric Assay Kit - Item No. 700610
- ChREBP Transcription Factor Assay Kit - Item No. 10006909
- Cortisol EIA Kit - Item No. 500360
- DPP (IV) Inhibitor Screening Assay Kit - Item No. 700210
- FABP4 (human) EIA Kit - Item No. 10007614
- Glucose Uptake Cell-Based Assay Kit - Item No. 600470
- Glycogen Assay Kit - Item No. 700480
- Hemoglobin Colorimetric Assay Kit - Item No. 700540
- β-Hydroxybutyrate (Ketone Body) Fluorometric Assay Kit - Item No. 700740
- Insulin (rat) EIA Kit - Item No. 589501
- D-Lactate Assay Kit - Item No. 700520
- L-Lactate Assay Kit - Item No. 700510
- Leptin (human) EIA Kit - Item No. 500010
- Leptin Receptor (human) EIA Kit - Item No. 10007608
- Lipase Activity Assay Kit - Item No. 700640
- NAD/NADH Cell Based Assay Kit - Item No. 600480
- Pyruvate Kinase Activity Assay Kit - Item No. 700760
- SREBP-2 Cell-Based Translocation Assay Kit - Item No. 10009239
- Urea Fluorometric Assay Kit - Item No. 700620
- Uric Acid Assay Kit - Item No. 700320

For a complete list of related products please visit: [www.caymanchem.com/catalog/10009582](http://www.caymanchem.com/catalog/10009582)
Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer’s exclusive remedy and Cayman’s sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman’s option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.