Glucose Assay Kit

Item No. 10009582

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity
10010098	Glucose Assay Standard	1 vial
10010099	Glucose Assay Buffer	1 vial
10010100	Glucose Enzyme Mixture (25X)	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) 975-3999. 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 capable of measuring absorbance between 500-520 nm
2. Adjustable pipettes and a repeat pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable
4. Test tubes, 12 x 75 mm and 18 x 150 mm
5. Incubator capable of incubating tubes at 37°C

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 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).[4]

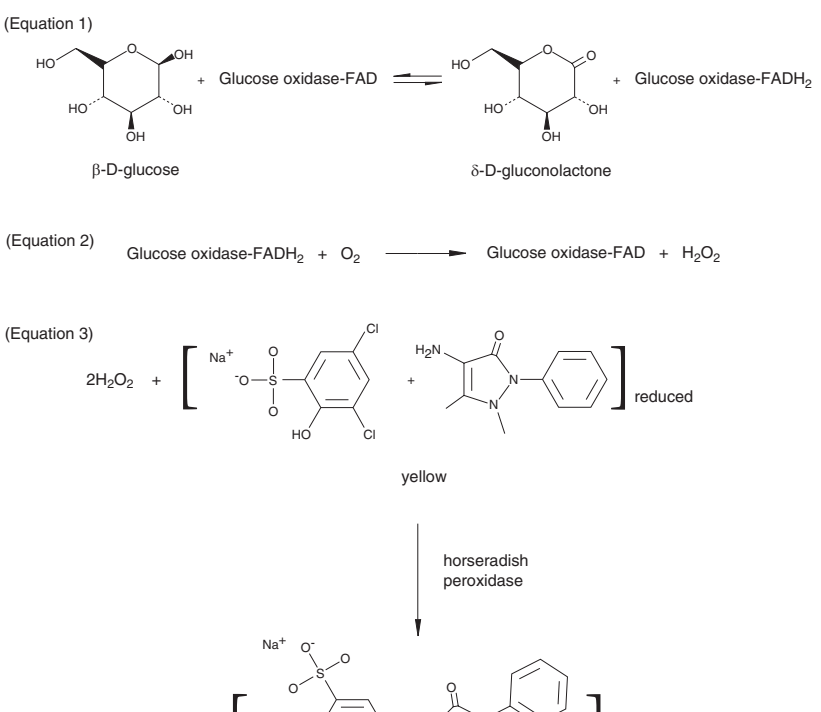


Figure 1. Biochemistry of the Glucose Assay

PRE-ASSAY PREPARATION

Reagent Preparation

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

Each vial contains 800 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. Glucose Assay Buffer - (Item No. 10010099)

The vial contains 65 ml of 100 mM PBS, pH 7.0. *NOTE: This solution should be thawed and stored at 4°C.* This solution is used to prepare the diluted Glucose Standards and for the final dilution of the Enzyme Mixture. One vial of Assay Buffer is sufficient to evaluate two 96-well plates.

3. Glucose Enzyme Mixture (25X) - (Item No. 10010100)

The vial contains a lyophilized enzyme mixture. Reconstitute 1 vial with 500 µl of 4°C UltraPure water. Transfer the reconstituted solution to an 18 x 150 mm test tube. Add 12 ml of Assay Buffer to the reconstituted solution and mix well. *NOTE: A portion of the 12 ml should be used to rinse any residual solution from the vial.* 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 48 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 while stored at -80°C.
3. Plasma does not need to be diluted 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. Serum does not need to be diluted 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, below. We suggest you record the contents of each well on the template sheet provided (see page 19).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

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 Glucose Assay Buffer should be thawed and equilibrated to 4°C. All reagents and samples must be equilibrated to 4°C before beginning the assay.
- The final volume of the assay is 150 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

For the determination of glucose in plasma or serum, prepare the Glucose Standards according to Table 1 (see page 11). For the determination of glucose in urine, prepare the Glucose Standards according to Table 2 (see page 12). Take eight clean 12 x 75 mm test tubes and label them A-H. Add the amount of Glucose Standard and Assay Buffer to each tube as described in Table 1 or 2.

Tube	Glucose Standard (µl) (1,000 mg/dl)	Assay Buffer (µl)	Glucose Concentration (mg/dl)
A	0	200	0
B	2.5	197.5	12.5
C	5	195	25
D	10	190	50
E	20	180	100
F	30	170	150
G	40	160	200
H	50	150	250

Table 1. Glucose standards to be assayed along with plasma and serum samples.

Tube	Glucose Standard (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 2. Glucose standards to be assayed along with urine samples.

*Dilute 100 µl of the glucose stock with 900 µl of Glucose Assay Buffer prior to setting up the glucose dilutions in Table 2.

Performing the Assay

1. Add 5 µl of sample or standard to appropriately labeled tubes.
2. Add 500 µl of the (1X) Enzyme Mixture forcefully down the side of each tube. Tap tubes a couple of times to mix thoroughly.
3. Place tubes in a 37°C incubator for 10 minutes.
4. After 10 minutes, remove tubes from incubator.
5. Load 150 µl (in duplicate) from each tube to the 96-well plate.
6. 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 or Table 2, pages 11 or 12).
4. Calculate the values of glucose for each sample from the standard curve. Examples of the glucose standard curves are shown in Figures 3 and 4, see pages 14-15.

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

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 0-250 mg/dl.

Representative Glucose Standard Curves

The standard curves presented here are examples 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.

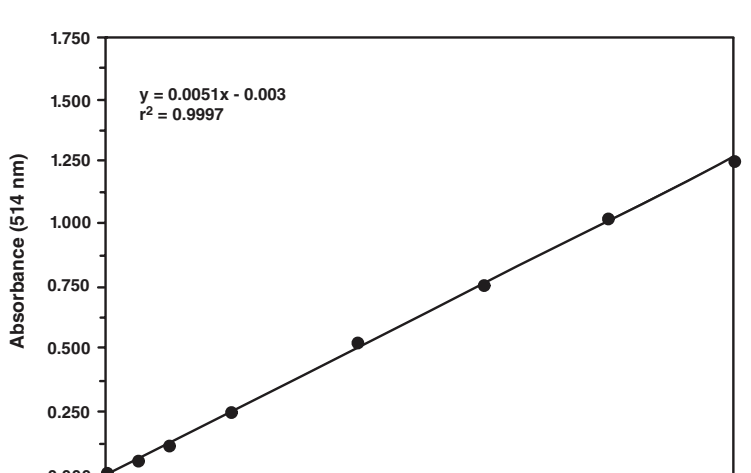


Figure 3. Glucose standard curve for plasma or serum

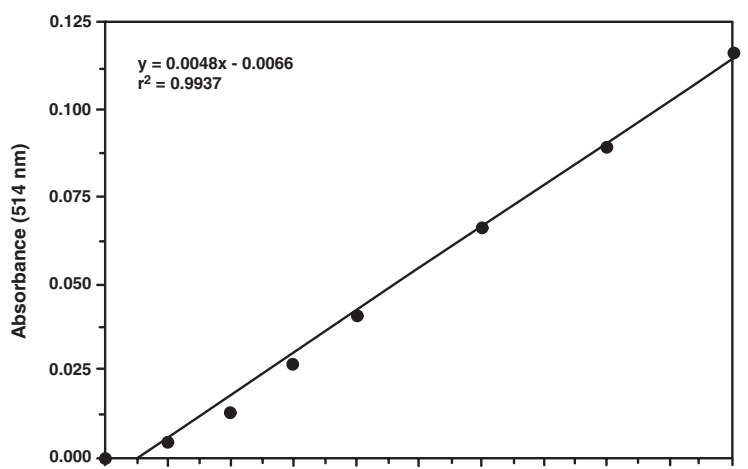


Figure 4. Glucose standard curve for urine

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 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

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).

Related Products

Acetylation Stimulating Protein (human) EIA Kit - Item No. 10008491
Adipogenesis Assay Kit - Item No. 10006908
Adiponectin (human) EIA Kit - Item No. 500641
Adiponectin (human) EIA Kit (HS) - Item No. 10007619
Adiponectin (mouse) EIA Kit - Item No. 10007620
AFABP (human) EIA Kit - Item No. 10007614
AgRP (human) EIA Kit - Item No. 10007615
ChREBP Transcription Factor Assay Kit - Item No. 10006909
Coenzyme A Assay Kit - Item No. 700440
Cortisol EIA Kit - Item No. 500360
Cortisol Express EIA Kit - Item No. 500370
D-Lactate Assay Kit - Item No. 700520
DPP (IV) Inhibitor Screening Assay Kit - Item No. 700210
Ghrelin (human acylated) EIA Kit - Item No. 10006306
Ghrelin (human unacylated) EIA Kit - Item No. 10008952
Ghrelin (rat acylated) EIA Kit - Item No. 10006307
Ghrelin (rat unacylated) EIA Kit - Item No. 10008953
Insulin (rat) EIA Kit - Item No. 589501
Leptin (human) EIA Kit - Item No. 500600
Leptin (mouse/rat) EIA Kit - Item No. 10007609
Leptin Receptor (human) EIA Kit - Item No. 10007608
L-Lactate Assay Kit - Item No. 700510
Pyruvate Assay Kit - Item No. 700470
Resistin (mouse) EIA Kit - Item No. 10005726
Resistin (rat) EIA Kit - Item No. 10007612
SREBP-2 Cell-Based Translocation Assay Kit - Item No. 10009239
Uric Acid Assay Kit - Item No. 700320

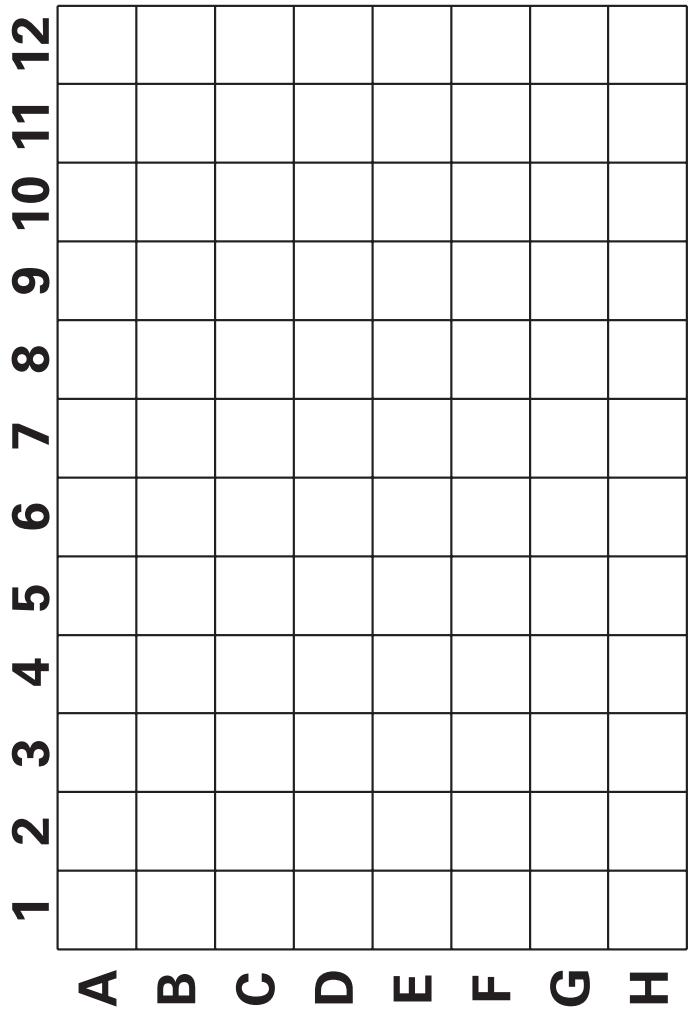
Warranty and Limitation of Remedy

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.



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