EZ- Glucose Assay Kit

Metabolism assay kit

(Colorimetric/Fluorometric)

Cat. No. DG-GCS100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

▪ Product Description

Glucose ($C_6H_{12}O_6$, FW: 180.16) is a primary biological fuel used to generate ATP, a universal energy molecule. It is also a key indicator of various metabolic disorders, making its measurement crucial for research and drug discovery.

The EZ-Glucose Assay Kit enables direct measurement of glucose in diverse biological samples, including serum, plasma, other body fluids, food and growth media. The Glucose Enzyme Mix specifically oxidizes glucose, producing by-products that react with a probe to generate absorbance (O.D 570 nm) and fluorescence (Ex/Em = 535/590 nm) signals. This method is rapid, simple, and sensitive, making it ideal for high-throughput sample processing. It is suitable for monitoring glucose consumption during protein expression or measuring glucose levels in fermentation processes.

▪ Contents and Storage Conditions

Component	100 assay	Storage
Glucose Assay buffer	25㎖	-20℃
Glucose Enzyme mix (Lyophilized)	1 vial	-20℃
Glucose Probe	200㎕	-20℃
Glucose Standard (100mM)	100㎕	-20℃

* This product is for research use only and must not be used for human or diagnostic purposes.

* "100 assays" means the kit has enough reagents to test about 100 wells in a 96-well plate. However, the actual number of samples you can test may be less because you'll need to use sample requirements to decide how many kits you need.

* If your sample's value is too high and falls outside the top range of the standard curve, dilute the sample so it fits within the proper range for accurate results.

▪ Sample type

This kit can measure glucose levels in various biological samples such as serum, plasma, urine, food, and culture media. It is also suitable for analyzing carbohydrate metabolism processes.

▪ Preparation of Reagent

Component	Preparation	Storage and Stability
Glucose Assay buffer	Before use, ensure that all reagents are fully thawed to room temperature. Using cold buffers may reduce enzyme activity and affect the results.	Store any remaining solution at -20℃ after use.
Glucose Enzyme mix (Lyophilized)	Dissolve the Enzyme Mix in 220 ㎕ of Assay Buffer per vial. Mix thoroughly by pipetting up and down until completely dissoloved.	The dissolved solution can be aliquoted and stored at -20℃ but should be used within 2 months.
Glucose Probe	Before use, ensure the solution is fully thawed to room temperature.	Protect from light and store at -20℃.
Glucose Standard	Before use, ensure the solution is fully thawed to room temperature.	Any remaining solution after use can also be stored at -20℃ but must be used within 2 months.

▪ General Protocol

1. Sample preparation

Add 2 ~ 50 ㎕ of the sample to a 96-well plate. Adjust the final volume in each well to 50 ㎕ using Assay Buffer. Perform the experiment with at least two replicates (n≥2).

1) Urine

- Use directly without preparation.

2) Serum

- Use 0.5 ~ 2.0 ㎕ per assay. Normal serum contains ~5 nmol/㎕ glucose.

3) Saliva

- Centrifuge at 14,000 rpm for 5 minutes and use the supernatant.

4) Milk

① Mix 600 ㎕ of milk with 100 ㎕ of 6N HCl, centrifuge at 14,000 rpm for 5 minutes, and collect the supernatant.

② Neutralize by adding 170 ㎕ of NaOH per ㎖, centrifuge again, and use the supernatant.

5) Unknown of First-Time Samples

- Perform a preliminiary test to ensure the measured value falls within the range of the standard curve.

6) Samples with High Background

- Prepare a sample background control using the same amount of sample without any reaction mix.

7) Spike Test

- Add 4 nmol of glucose standard to confirm accurate measurement.

8) Enzyme-Containing Samples

- Deproteinize using a 10 kDa spin column to prevent glucose consumption.

2. Standard preparation

1) Colorimetric method

(1) Prepare a 1 mM Glucose Standard Solution by mixing 10 ㎕ of 100 mM Glucose Standard with 990 ㎕ of Assay Buffer.

(2) Add 0, 2, 4, 6, 8 and 10 ㎕ of the 1mM Glucose Standard to wells in a 96-well plate.

(3) Adjust the final volume in each well to 50 ㎕ with Assay Buffer. This will create a standard set with 0, 2, 4, 6, 8 and 10 nmol/well.

Standard No.	Volume of 1mM Glucose Standard	Volume of Assay buffer	Final standard volume in well	Final standard Glucose Conc. (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	2
3	4 ㎕	46 ㎕	50 ㎕	4
4	6 ㎕	44 ㎕	50 ㎕	6
5	8 ㎕	42 ㎕	50 ㎕	8
6	10 ㎕	40 ㎕	50 ㎕	10

* It is recommended to prepare and measure the standards with each experiment.

2) Fluorometric method

(1) Prepare a 1mM Glucose Standard Solution by mixing 10 µℓ of 100 mM Glucose Standard with 990 µℓ of Assay Buffer.

(2) To prepare a 0.1 mM Glucose Standard Solution, mix 50 µℓ of the 1mM Glucose Standard Solution with 450 µℓ of Assay Buffer.

(3) Dispense 0, 2, 4, 6, 8 and 10 µℓ of the 0.1 mM Glucose Standard Solution into wells of a 96-well plate.

(4) Adjust the volume in each well to 50 µℓ with Assay buffer. This will create a standard set with 0, 0.2, 0.4, 0.6, 0.8 and 1.0 nmol of glucose per well.

Standard No.	Volume of 0.1mM Glucose Standard	Volume of Assay buffer	Final standard volume in well	Final standard Glucose Conc. (nmol/well)
1	0 µℓ	50 µℓ	50 µℓ	0
2	2 µℓ	48 µℓ	50 µℓ	0.2
3	4 µℓ	46 µℓ	50 µℓ	0.4
4	6 µℓ	44 µℓ	50 µℓ	0.6
5	8 µℓ	42 µℓ	50 µℓ	0.8
6	10 µℓ	40 µℓ	50 µℓ	1.0

* It is recommended to prepare and measure the standards with each experiment.

3. Reaction mixture preparation

The provided volumes are for single assay. Calculate the total number of assays you plan to perform and prepare the reaction mix with extra to ensure sufficient volume for the experiment.

1) Colorimetric method:

Components	Reaction mixture	Background mixture
Glucose Assay buffer	46µℓ	48µℓ
Glucose Enzyme mix	2µℓ	-
Glucose Probe	2µℓ	2µℓ
ToTal	50µℓ	50µℓ

2) Fluorometric method:

Components	Reaction mixture	Background mixture
Glucose Assay buffer	47.6㎕	49.6㎕
Glucose Enzyme mix	2㎕	-
Glucose Probe	0.4㎕	0.4㎕
ToTal	50㎕	50㎕

4. Add 50㎕ of the reaction mixture to each well containing the Glucose Standard and samples using a multi-channel pipette, then mix well.

* If a sample background control is prepared, add 50 ㎕ of the background mixture to the corresponding wells.

5. Incubate the plate at 37℃ in the dark for 30 minutes, then shake gently and measure with a microplate reader.

1) Colorimetric : 570 nm
2) Fluorometric: (Excitation/Emission): 535 nm / 595 nm

▪ Calculation

1. Subtract the blank value (Standard 1) from all measurement values.
2. Calculate the average of the duplicate measurements for each standard and sample well.
3. Create a standard curve by plotting the Glucose Standard concentrations against the O.D 570nm values.
4. Use the standard curve to determine the glucose content in the samples by applying their measured values to the curve.
   * If a background control is set, subtract the background control value from the sample measurement. Use the corrected value to calculate the glucose content based on the standard curve.

5. Based on the glucose amount calculated in step 4, determine the glucose concentration in the sample using the following formula:

$$\boldsymbol{Glucose\ Concentration\ (C) = \frac{B}{V} X\ D\ (nmol/\mu\ell\ or\ mM)}$$

B: Glucose amount in the well (nmol), determined from the standard curve.

V: Volume of the sample added to the well ($\mu\ell$).

D: Dilution factor of the sample (e.g., for a 2-fold dilution, use D = 2)

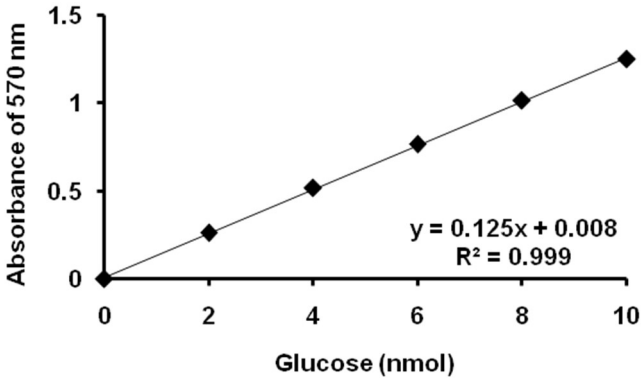

Fig. Glucose standard curve. Assay was performed following the kit protocol.

- Spike sample: When a component in the sample may interfere with the reaction (e.g., the actual glucose content is 2 ng, but the result shows only 1.6 ng due to interference), a spike test can correct for this effect.

- When using a spike sample in this experiment, the glucose concentration in the sample can be calculated using the following formula.

$$\boldsymbol{Glucose\ amount\ in\ the\ sample\ (B) = \frac{O.D\ 1}{O.D\ 2 - O.D\ 3}\ X\ Glucose\ spike\ (nmol)}$$

* O.D 1: O.D value of the sample(blank-corrected).

* O.D 2: O.D value of the spiked sample(blank-corrected).

* O.D 3. O.D value of the sample(blank-corrected, same as O.D 1)

* Glucose spike: The amount of glucose added to the spiked sample(nmol).

This formula adjusts for interference and provides the actual glucose amount in the sample.