Glucose Assay Kit

(Catalog #K606-100; 100 assays; Store at -20°C.)

I. Introduction:

Glucose ($C_6H_{12}O_6$; FW: 180.16) is a very important fuel source to generate universal energy molecules ATP. Glucose level is a key diagnostic parameter for many metabolic disorders. Measurement of glucose can be very important in both research and drug discovery processes. The Glucose Assay Kit provides direct measurement of glucose in various biological samples (e.g., serum, plasma, body fluid, food, growth medium, etc.). Glucose Enzyme Mix specifically oxidizes glucose to generate a product which reacts with a dye to generate color (λ = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The generated color and fluorescence is proportionally to the glucose amount. The method is rapid, simple, sensitive, and suitable for high throughput. The assay is also suitable for monitoring glucose level during fermentation and glucose feeding in protein expression processes. The kit detects 1-10000µM glucose samples.

II. Kit Contents

Component	K606-100	Cap Code	Part No.
Glucose Assay Buffer	25 ml	WM	K606-100-1
Glucose Probe (lyophilized)	1 vial	Red	K606-100-2
Dimethylsulfoxide (DMSO; Dried)	0.4 ml	Brown	K606-100-3
Glucose Enzyme Mix (lyophilized)	1 vial	Green	K606-100-4
Glucose Standard (100 nmol/µl)	100 µl	Yellow	K606-100-5

III. Storage and Handling:

Store kit at –20°C, protect from light. Warm the Glucose Assay Buffer to room temperature and briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Preparation:

Glucose Probe: Dissolve in 220 µl DMSO (provided) before use. Store at –20°C, protect from light and moisture. Use within two months.

Glucose Enzyme Mix: Dissolve in 220 µl Glucose Assay Buffer. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.

V. Glucose Assay Protocol:

1. Standard Curve Preparations:

For colorimetric assay, dilute the Glucose Standard to 1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Glucose Standard.

For the fluorometric assay, dilute the Glucose Standard solution to 0.1 nmol/µl by adding 10 µl of the Glucose Standard to 990 µl of Glucose Assay Buffer, mix well. Then take 20 µl into 180 µl of Glucose Assay Buffer. Mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Glucose Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well of the Glucose Standard.

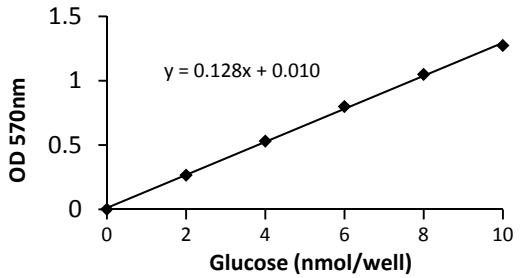
2. **Sample Preparations:** Prepare test samples in 50 µl/well with Glucose Assay Buffer in a 96-well plate. If using serum sample, serum (0.5-2 µl/assay. Normal serum contains ~5 nmol/µl glucose) can be directly diluted in the Glucose Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.
3. **Glucose Reaction Mix:** Mix enough reagents for the number of assays to be performed: For each well, prepare a total 50 µl Reaction Mix containing:

   46 µl Glucose Assay Buffer
   2 µl Glucose Probe*
   2 µl Glucose Enzyme Mix

   ***Note:** The fluorometric assay is 10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction will decrease the background reading significantly to increase the detection sensitivity.
4. Mix well. Add 50 µl of the Reaction Mix to each well containing the Glucose Standard and test samples. Mix well.
5. Incubate the reaction for 30 minutes at 37°C, protect from light.
6. Measure O.D. 570nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microplate reader.
7. **Calculations:** Correct background by subtracting the value derived from the 0 glucose control from all readings (Note: The background reading can be significant and must be subtracted from sample readings). Glucose concentrations of the test samples can then be calculated.

   C = Sa/Sv (nmol/µl or µmol/ml, or mM)

   Where Sa is sample amount (in nmol) from standard curve.
   Sv is sample volume (in µl) added into the sample wells.
   Glucose Molecular Weight 180.16.



VI. RELATED PRODUCTS:

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