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Product Information

Glucose Assay Kit

Catalog Number **MAK263**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Glucose is a primary energy source that naturally occurs in its free state in fruits and other plant parts. Abnormal glucose levels have been associated with several metabolic dysfunctions such as hypoglycemia, hyperglycemia, and diabetes mellitus. Measurements of glucose levels in tissues and body fluids (such as blood and urine) are often used for the diagnosis of glucose–related disorders. Glucose levels are also monitored to check the efficacy of therapeutics such as insulin and sulfonylureas in type 2 diabetics.[1,2]

The Glucose Assay Kit provides direct measurement of glucose in various biological samples, including serum, plasma, food, or growth medium. In this kit, glucose is oxidized to generate a colorimetric (570 nm)/ fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the amount of glucose present. The kit is able to detect 1-10,000 μM of glucose in various samples.

This simple, sensitive, high throughput assay is also suitable for monitoring glucose levels during fermentation and glucose feeding in protein expression processes.

Components

The kit is sufficient for 100 assays in 96 well plates.

Glucose Assay Buffer Catalog Number MAK263A	25 mL
Glucose Probe, in DMSO Catalog Number MAK263B	0.2 mL
Glucose Enzyme Mix Catalog Number MAK263C	1 vl
Glucose Standard, 100 nmole/μL Catalog Number MAK263D	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Glucose Assay Buffer – Allow buffer to come to room temperature before use.

Glucose Probe – Warm to room temperature to thaw the solution prior to use. Store protected from light and moisture at –20 °C. Use within 2 months. Upon thawing, the Glucose Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Glucose Probe Solution 5 to 10-fold with Glucose Assay Buffer just prior to use. This will reduce the background of the fluorescence assay.

Glucose Enzyme Mix – Reconstitute each with 220 μL of Glucose Assay Buffer. Mix well by pipetting (do not vortex), then aliquot and store, protected from light and moisture, at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Glucose Standards for Colorimetric Detection
Dilute 10 µL of the 100 nmole/µL Glucose Standard with 990 µL of the Assay Buffer to prepare a 1 nmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Glucose Assay Buffer to each well to bring the volume to 50 µL.

Glucose Standards for Fluorometric Detection
Prepare a 1 nmole/µL solution as for the colorimetric assay. Dilute 20 µL of the 1 nmole/µL solution with 180 µL of the Glucose Assay Buffer to prepare a 0.1 nmole/µL solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Glucose Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well). Samples may be assayed directly.

Add 2–50 µL samples into wells of a 96 well plate. Limit serum sample volume to 0.5–2 µL/assay (normal serum contains ~5 nmole/µL glucose).

Bring samples to a final volume of 50 µL with Assay Buffer.

Note: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

Metabolites found in biological samples can interfere with the assay. If interference is observed in the diluted samples, prepare parallel sample well(s) as sample background control(s) by omitting the Glucose Enzyme Mix.

For samples with high protein content, deproteinize using a 10 kDa MWCO spin filter.

To ensure accurate determination of glucose in the test samples or for samples having low concentrations of glucose, spike samples with a known amount of Glucose Standard (e.g., 4 nmole).

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Samples and Positive Control	Background Control Mix
Glucose Assay Buffer	46 µL	48 µL
Glucose Probe	2 µL	2 µL
Glucose Enzyme Mix	2 µL	–

2. Add 50 µL of the Master Reaction Mix to each sample and positive control well. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 30 minutes at 37 °C. Protect the plate from light during the incubation.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (assay blank) Glucose Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Glucose standards to plot a standard curve. The amount of glucose present in the samples may be determined from the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Glucose

$$S_a/S_v = C$$

S_a = Amount of Glucose in the unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of Glucose in sample

Glucose molecular weight: 180.16 g/mole

Sample Calculation

Amount of Glucose (S_a) = 5.84 nmole (from standard curve)
Sample volume (S_v) = 50.0 μL

Concentration of Glucose in sample

$$5.84 \text{ nmole}/50.0 \text{ μL} = 0.117 \text{ nmole}/\text{μL}$$

$$0.117 \text{ nmole}/\text{μL} \times 180.16 \text{ ng/nmole} = 21.1 \text{ ng}/\text{μL}$$

Concentration of Glucose in spiked samples

For spiked samples, calculate the amount of glucose in the sample wells after correcting for the Sample Blank and background.

$$C = \frac{S_p \times A_s}{(A_{sp} - A_s) \times S_v}$$

Where:
S_p = Known amount of Glucose Standard spiked in well (nmole)
A_s = Corrected sample reading (A_{570}) (unspiked well)
A_{sp} = Corrected sample + spike reading (A_{570})
S_v = Sample volume (μL) added into the well
C = Concentration of Glucose in sample (nmole/μL)

Glucose molecular weight: 180.16 g/mole

Sample Calculation

Amount of Glucose (S_a) = 5.84 nmole (from standard curve)
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Concentration of Glucose in sample

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References

1. Golden, S.H., and Sapir, T., Methods for insulin delivery and glucose monitoring in diabetes: summary of a comparative effectiveness review. J. Manag. Care Pharm., **18**(6 suppl.), S1–17 (2012).
2. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet, **352**(9131), 837–853 (1998).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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