**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 (\(\lambda_{ex} = 535/\lambda_{em} = 587\) 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).

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**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**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Samples and Positive Control</th>
<th>Background Control Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Assay Buffer</td>
<td>46 μL</td>
<td>48 μL</td>
</tr>
<tr>
<td>Glucose Probe</td>
<td>2 μL</td>
<td>2 μL</td>
</tr>
<tr>
<td>Glucose Enzyme Mix</td>
<td>2 μL</td>
<td>–</td>
</tr>
</tbody>
</table>

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

\[
\frac{S_a}{S_v} = C
\]

\(S_a\) = Amount of Glucose in the unknown sample (nmole) from standard curve

\(S_v\) = Sample volume (\(\mu\)L) 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 \(\mu\)L

Concentration of Glucose in sample

\[
\frac{5.84 \text{ nmole}}{50.0 \text{ \(\mu\)L}} = 0.117 \text{ nmole/\(\mu\)L}
\]

\[
0.117 \text{ nmole/\(\mu\)L} \times 180.16 \text{ ng/nmole} = 21.1 \text{ ng/\(\mu\)L}
\]

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.

\[
C = \frac{S_a \times A_{sb}}{(A_{sp} - A_s) \times S_v}
\]

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 (\(\mu\)L) added into the well

\(C\) = Concentration of Glucose in sample (nmole/\(\mu\)L)

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References

## Troubleshooting Guide

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