Product Information

Glucose Oxidase Activity Assay Kit

Catalog Number MAK097
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Glucose Oxidase (GOx, EC 1.1.3.4) is an enzyme found in insects, fungi, and bacteria that catalyzes the oxidation of D-glucose to D-gluconolactone. GOx is widely used in the food, beverage, chemical, and pharmaceutical industries.

The Glucose Oxidase Activity Assay Kit provides a simple and direct procedure for measuring GOx activity in a variety of biological samples. GOx activity is determined by a coupled enzyme assay, in which GOx oxidizes D-glucose resulting in the production of hydrogen peroxide (H₂O₂) that reacts with a probe, generating a colorimetric (570 nm)/fluorometric (ex = 535/ems = 587 nm) product, proportional to the GOx present. One unit of GOx is defined as the amount of enzyme that generates 1.0 μmole of H₂O₂ per minute at 37 °C.

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

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Concentration</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOx Assay Buffer</td>
<td>28 mL</td>
<td>MAK097A</td>
</tr>
<tr>
<td>Fluorescent Peroxidase Substrate, in DMSO</td>
<td>0.2 mL</td>
<td>MAK097B</td>
</tr>
<tr>
<td>GOx Substrate</td>
<td>1 mL</td>
<td>MAK097C</td>
</tr>
<tr>
<td>GOx Developer</td>
<td>1 vl</td>
<td>MAK097D</td>
</tr>
<tr>
<td>GOx Positive Control</td>
<td>1 vl</td>
<td>MAK097E</td>
</tr>
<tr>
<td>H₂O₂ Standard, 0.88 M</td>
<td>0.1 mL</td>
<td>MAK097F</td>
</tr>
</tbody>
</table>

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.

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

Fluorescent Peroxidase Substrate – Allow reagent to come to room temperature before use. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C. Upon thawing, the Fluorescent Peroxidase Substrate is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Fluorescent Peroxidase Substrate 5 to 10-fold with GOx Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

GOx Developer and GOx Positive Control –
Reconstitute each in 220 μL of GOx Assay Buffer. Mix well by pipetting (don’t vortex), then aliquot each and store at –20 °C. Use within two months of reconstitution and keep cold while in use.

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. Use ultrapure water for the preparation of standards.

H₂O₂ Standards for Colorimetric Detection
Dilute 10 μL of the 0.88 M H₂O₂ with 870 μL of water to prepare a 10 mM standard solution. Further dilute the 10 mM standard to 0.5 mM by diluting 50 μL of the 10 mM standard solution with 950 μL of GOx Assay Buffer. Add 0, 2, 4, 6, 8, 10 μL of the 0.5 mM standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. Add GOx Assay Buffer to each well to bring the volume to 50 μL.

H₂O₂ Standards for Fluorometric Detection
Prepare a 0.5 mM standard solution as for the colorimetric assay. Dilute 10 μL of the 0.5 mM standard solution with 90 μL of the GOx Assay Buffer to make a 50 μM standard solution. Add 0, 2, 4, 6, 8, 10 μL of the prepared 50 μM standard solution into a 96 well plate, generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add GOx 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).

Tissue (10 mg) or cells (1 × 10⁶) should be rapidly homogenized with 100–200 μL of GOx Assay Buffer. Centrifuge at 15,000 × g for 10 minutes to remove insoluble materials. Bring samples to a final volume of 50 μL with GOx Assay Buffer.

Serum samples can be directly added to wells. Add 1–50 μL samples into wells of a 96 well plate. Bring samples to final volume of 50 μL with GOx Assay Buffer.

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

Notes: High background may be a problem for some samples. To remove the effect of background, a sample blank may be set up for each sample by omitting the GOx Substrate.

For the positive control (optional), add 2–10 μL of the GOx Positive Control to wells.

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

Table 1. Reaction Mixes

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Samples and Standards</th>
<th>Blank Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOx Assay Buffer</td>
<td>36 μL</td>
<td>46 μL</td>
</tr>
<tr>
<td>GOx Developer</td>
<td>2 μL</td>
<td>2 μL</td>
</tr>
<tr>
<td>Fluorescent Peroxidase Substrate</td>
<td>2 μL</td>
<td>2 μL</td>
</tr>
<tr>
<td>GOx Substrate</td>
<td>10 μL</td>
<td>–</td>
</tr>
</tbody>
</table>

2. Add 50 μL of the appropriate Reaction Mix to each well. Mix well using a horizontal shaker or by pipetting.

3. After 5 minutes, take the initial measurement (T_initial). For colorimetric assays, measure the absorbance at 570 nm (A_570_initial). For fluorometric assays, measure fluorescence intensity (FLU_initial, λ_ex = 535/λ_em = 585 nm).

4. Incubate the plate at 37 °C taking measurements (A_570 or FLU) every 2–3 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (Colorimetric – 5 nmole/well or fluorometric – 0.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final measurement [(A_570)final or FLU_final] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_final.

Note: It is essential the final measurement falls within the linear range of the standard curve.
Results
Calculations
Correct for the background by subtracting the final measurement \([A_{570}]_{\text{final}}\) or \([\text{FLU}]_{\text{final}}\) obtained for the 0 (blank) standard from the final measurement \([A_{570}]_{\text{final}}\) or \([\text{FLU}]_{\text{final}}\) of the standards. Plot the H$_2$O$_2$ standard curve.

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

Calculate the change in measurement from \(T_{\text{initial}}\) to \(T_{\text{final}}\) for samples and sample blanks.

\[
\Delta A_{570} = (A_{570})_{\text{final}} - (A_{570})_{\text{initial}} \\
\Delta \text{FLU} = \text{FLU}_{\text{final}} - \text{FLU}_{\text{initial}}
\]

Also, subtract the Sample Blank \(\Delta\) measurement value from the sample \(\Delta\) measurement values. Compare the \(\Delta\) measurement value (\(\Delta A_{570}\) or \(\Delta\text{FLU}\)) of each sample to the standard curve to determine the amount of H$_2$O$_2$ generated by the oxidase assay between \(T_{\text{initial}}\) and \(T_{\text{final}}\) (B).

The Glucose Oxidase activity of a sample may be determined by the following equation:

\[
\text{GOx Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}
\]

\(B = \text{Amount (n mole) of H}_2\text{O}_2 \text{ generated between } T_{\text{initial}} \text{ and } T_{\text{final}}\)

\(\text{Reaction Time} = T_{\text{final}} - T_{\text{initial}} \) (minutes)

\(V = \text{sample volume (mL) added to well}\)

GOx activity reported as n mole/min/mL = milliunit/mL, where one unit of GOx is defined as the amount of enzyme that generates 1.0 $\mu$mole of H$_2$O$_2$ per minute at 37 °C.
## Troubleshooting Guide

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<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
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</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>
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<td></td>
<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if samples will be used multiple times</td>
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<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>