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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 (H_2O_2) that reacts with a probe, generating a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 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 H_2O_2 per minute at 37 °C.

Components

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

GOx Assay Buffer Catalog Number MAK097A	28 mL
Fluorescent Peroxidase Substrate, in DMSO Catalog Number MAK097B	0.2 mL
GOx Substrate Catalog Number MAK097C	1 mL
GOx Developer Catalog Number MAK097D	1 vl
GOx Positive Control Catalog Number MAK097E	1 vl
H_2O_2 Standard, 0.88 M Catalog Number MAK097F	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.

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.

H_2O_2 Standards for Colorimetric Detection
Dilute 10 µL of the 0.88 M H_2O_2 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.

H_2O_2 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 (1×10^6) 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

Reagent	Samples and Standards	Blank Sample
GOx Assay Buffer	36 µL	46 µL
GOx Developer	2 µL	2 µL
Fluorescent Peroxidase Substrate	2 µL	2 µL
GOx Substrate	10 µL	–

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 ($FLU_{initial}$, $\lambda_{ex} = 535/\lambda_{em} = 585$ 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 [$(A_{570})_{final}$ or FLU_{final}] obtained for the 0 (blank) standard from the final measurement [$(A_{570})_{final}$ or FLU_{final}] of the standards. Plot the H_2O_2 standard curve.

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

Calculate the change in measurement from $T_{initial}$ to T_{final} for samples and sample blanks.

$$\Delta A_{570} = (A_{570})_{final} - (A_{570})_{initial}$$

or

$$\Delta FLU = FLU_{final} - FLU_{initial}$$

Also, subtract the Sample Blank Δmeasurement value from the sample Δmeasurement values. Compare the Δmeasurement value (ΔA_{570} or ΔFLU) of each sample to the standard curve to determine the amount of H_2O_2 generated by the oxidase assay between $T_{initial}$ and T_{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 = Amount (nmole) of H_2O_2 generated between $T_{initial}$ and T_{final}

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

V = sample volume (mL) added to well

GOx activity reported as nmole/min/mL = milliunit/mL, where one unit of GOx is defined as the amount of enzyme that generates 1.0 μmole of H_2O_2 per minute at 37 °C.

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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