Technical Bulletin

# Glucose Oxidase Activity Assay Kit

**Catalogue number MAK501**

## Product Description

Glucose oxidase catalyzes the oxidation of glucose from D-glucose to D-glucono-δ-lactone. Physiologically, it aids in the breakdown of glucose into smaller metabolites. It is widely used in electrochemical glucose sensors designed for diabetes patients. Simple, direct, and high-throughput assays for measuring glucose oxidase activity find wide applications in research and drug discovery.

The glucose oxidase assay kit uses a single Working Reagent that combines the glucose oxidase reaction and color reaction in one step. The change in absorbance at 570 nm or fluorescence intensity at $\lambda_{Ex}$ =530 nm/$\lambda_{Em}$ =585 nm is directly proportional to glucose oxidase activity in the sample.

The linear detection range of the kit is 0.02 to 10 U/L glucose oxidase for colorimetric assays and 0.002 to 1.5 U/L for fluorometric assays. The kit is suitable for glucose oxidase activity determination in cell lysate, culture medium and other biological samples, as well as for studying the effects of drugs on glucose metabolism.

## Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK501A
- HRP Enzyme 120 µL
  Catalogue Number MAK501B
- 2 M Glucose 1.5 mL
  Catalogue Number MAK501C
- Dye Reagent 120 µL
  Catalogue Number MAK501D
- Standard (3% $H_2O_2$) 100 µL
  Catalogue Number MAK501E

## Equipment Required but Not Provided

- Pipetting devices and accessories
  (For Example., multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

## Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

## Preparation Instructions

Briefly centrifuge small vials prior to opening.
HRP Enzyme: During experiment, keep thawed Enzyme in a refrigerator or on ice. Vortex briefly before pipetting.
Equilibrate all other components to room temperature.

## Procedure

All samples and standards should be run in duplicate.

### Sample Preparation

1. Samples can be analyzed immediately after collection, or stored in aliquots at -20 °C.
2. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.
3. Transfer 20 µL of each Sample into separate wells of a clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay.

### Colorimetric Standard Curve Preparation:

1. Prepare 4.8 mM $H_2O_2$ by mixing 5 µL 3% $H_2O_2$ and 914 µL purified water.
2. Prepare 200 µM $H_2O_2$ by mixing 20 µL of the 4.8 mM $H_2O_2$ with 460 µL purified water.
3. Prepare Standards as described in the Table 1.

**Table 1.**
Preparation of $H_2O_2$ Colorimetric Standards

| Well | 200 µM Standard | Purified Water | $H_2O_2$ (µM) |
|---|---|---|---|
| 1 | 100 µL | 0 µL | 200 |
| 2 | 60 µL | 40 µL | 120 |
| 3 | 30 µL | 70 µL | 60 |
| 4 | 0 µL | 100µL | 0 |

4. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96-well plate

### Fluorometric Standard Curve Preparation

1. Dilute the Standards #1, #2, and #3 from Colorimetric Standard Curve Preparation 10X with purified water as shown in Table 2.

**Table 2.**
Preparation of $H_2O_2$ Fluorometric Standards

| Well | Standards (from Table 1) | | Purified Water | $H_2O_2$ (µM) |
|---|---|---|---|---|
| 1 | Standard #1 | 10 µL | 90 µL | 20 |
| 2 | Standard #2 | 10 µL | 90 µL | 12 |
| 3 | Standard #3 | 10 µL | 90 µL | 6 |
| 4 | N/A | 0 µL | 100 µL | 0 |

2. Mix well and transfer 20 µL of each Standard into separate wells of a black 96 well plate.

### Working Reagent Preparation

Mix enough reagent for the number of assays to be performed. For each Standard and Sample well, prepare 87 µL of Working Reagent according to Table 3.

**Table 3.**

Preparation of Working Reagents

| Reagent | Working Reagent |
|---|---|
| Assay Buffer | 75 µL |
| 2 M Glucose | 10 µL |
| HRP Enzyme | 1 µL |
| Dye reagent | 1 µL |

Transfer 80 µL Working Reagent into each reaction well. Tap plate to mix.

### Measurement

1. Read optical density ($OD_0$) at 570 nm (550-585 nm) or fluorescence intensity ($F_0$) at $\lambda_{Ex}$ =530 nm/$\lambda_{Em}$ =585 nm immediately.
2. Incubate 20 min at room temperature, and then read optical density ($OD_{20}$) at 570 nm (550-585 nm) or fluorescent intensity ($F_{20}$) at $\lambda_{Ex}$ =530 nm/$\lambda_{Em}$ =585 nm again.

## Results

1. Subtract blank $OD_{20}$ or $F_{20}$ (Std #4) from all the standard $OD_{20}$ or $F_{20}$ values.
2. Plot the ΔF or ΔOD against the standard concentrations. Determine the slope using linear regression.
3. Calculate the $\Delta OD_{Sample}$ or $\Delta F_{Sample}$ of all samples by subtracting $OD_0$ or $F_0$ from $OD_{20}$ or $F_{20}$ for each sample. Do the same for the blank (water, standard #4) to get $\Delta OD_{Blank}$ or $\Delta F_{Blank}$.
4. Calculate Glucose Oxidase concentration of samples using the below given equation:

Glucose Oxidase (U/L) =

$$\frac{\Delta R_{Sample} - \Delta R_{Blank}}{\text{Slope}\left(\mu M^{-1}\right)\cdot t} \times DF$$

Where:
$\Delta R_{Sample}$ = the change in optical density or fluorescent values of the sample
$\Delta R_{Blank}$ = the change in optical density or fluorescent values of the blank
Slope = the slope of the $H_2O_2$ standard curve.
t = the incubation time (20 minutes)
DF = the dilution factor.
Unit definition: 1 U/L of Glucose Oxidase catalyzes 1 µmole of $H_2O_2$ per minute at pH 7.0 and room temperature.

**Note** If the calculated sample glucose concentration is higher than 10 U/L in colorimetric assay or 1.5 U/L in fluorometric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor (DF). For samples with low Glucose Oxidase activity, the incubation time can be increased.

**Figure 1.**

Typical Glucose Oxidase Colorimetric Standard Curve

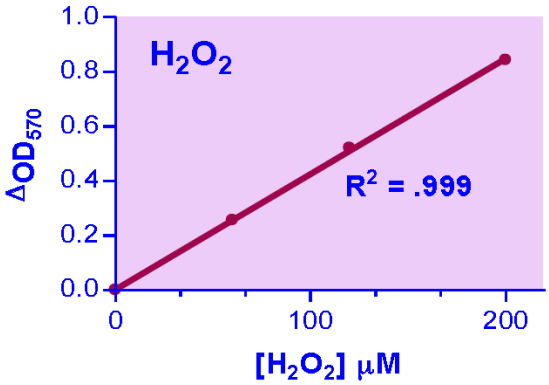
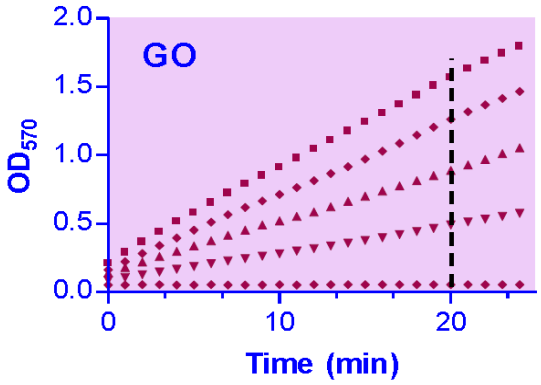



**Figure 2.**

Typical Colorimetric Glucose Oxidase $OD_{570}$ Readings




## References


1. J, Raba., *et al.,* Glucose Oxidase as an Analytical Reagent. *Critical Reviews in Analytical Chemistry* **25(1)**.,1-42 (1995).
2. JM, Harris., *et al*., Common Causes of Glucose Oxidase Instability in in vivo Biosensing: A Brief Review. *J Diabetes Sci Technol* **7(4)**.,1030-8 (2013).
3. S, Ferri., Review of glucose oxidases and glucose dehydrogenases., a bird's eye view of glucose sensing enzymes. *J Diabetes Sci Technol* **5(5)**., 1068-76 (2011).

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mak501pis Rev 08/23