



Glucose Oxidase Microplate Assay Kit

User Manual

Catalog # FTA0056

(Version 1.2D)

Detection and Quantification of Glucose Oxidase (GOD) Activity in
Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media
and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

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

The Glucose Oxidase Activity Microplate Assay Kit provides a simple and direct procedure for measuring GOD activity in a variety of biological samples. GOD activity is determined by a coupled enzyme assay, in which GOD oxidizes D-glucose resulting in the production of hydrogen peroxide (H_2O_2) that reacts with a probe.

II. KIT COMPONENTS

| Component | Volume | Storage |
|---------------------|------------|---------|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30 ml x 4 | 4 °C |
| Dye Reagent | Powder x 1 | 4 °C |
| Dye Reagent Diluent | 1 ml x 1 | 4 °C |
| Substrate | Powder x 1 | 4 °C |
| Enzyme | Powder x 1 | -20 °C |
| Standard (3 mmol/L) | 1 ml x 1 | 4 °C |
| Technical Manual | 1 Manual | |

Note:

Dye Reagent: add 1 ml Dye Reagent Diluent to dissolve before use.

Substrate: add 15 ml Assay Buffer to dissolve before use.

Enzyme: add 1 ml Assay Buffer to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 460 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm the Dye Reagent, Substrate, Enzyme to room temperature before use.

Add following reagents into the microplate:

| Reagent | Sample | Standard | Blank |
|--|-------------|-------------|-------------|
| Dye Reagent | 10 μ l | 10 μ l | 10 μ l |
| Substrate | 150 μ l | 150 μ l | 150 μ l |
| Enzyme | 20 μ l | 20 μ l | 20 μ l |
| Mix. | | | |
| Sample | 20 μ l | -- | -- |
| Standard | -- | 20 μ l | -- |
| Distilled water | -- | -- | 20 μ l |
| Mix, incubate for 2 minutes, measured at 460 nm and record the absorbance. | | | |

Note:

- 1) Perform 2-fold serial dilutions of the top standards to make the standard curve.
- 2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VI. CALCULATION

Unit Definition: One unit of GOD is defined as the enzyme that generates 1 μmol H_2O_2 per minute.

1. According to the protein concentration of sample

$$\begin{aligned}\text{GOD (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times C_{\text{Protein}}) / T \\ &= 1.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{GOD (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 1.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned}\text{GOD (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 1.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned}\text{GOD (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}}/T \\ &= 1.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

C_{Protein} : the protein concentration, mg/ml;

C_{Standard} : the concentration of Standard, 3 mmol/L = 3 $\mu\text{mol/ml}$;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Standard} : the volume of standard, 0.02 ml;

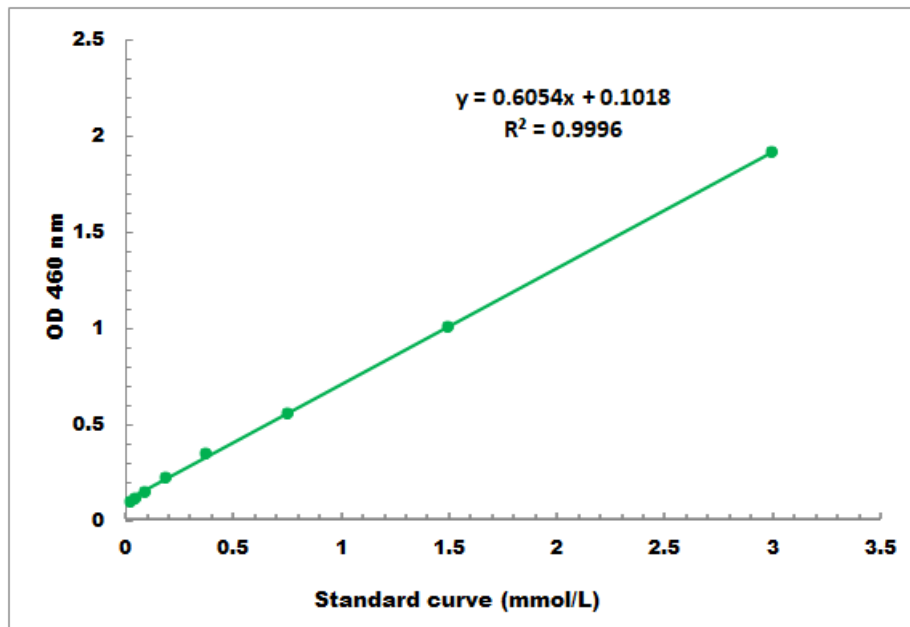
V_{Sample} : the volume of sample, 0.02 ml;

V_{Assay} : the volume of Assay buffer in sample preparation, 1 ml;

T: the reaction time, 2 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.03 mmol/L - 3 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES