



# Glucose Oxidase Microplate Assay Kit

## User Manual

Catalog # ASK1057

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<sub>2</sub>O<sub>2</sub>) 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 (5 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
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  $5 \times 10^6$  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 µl	10 µl	10 µl
Substrate	150 µl	150 µl	150 µl
Enzyme	20 µl	20 µl	20 µl
Mix.			
Sample	20 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Mix, incubate for 2 minutes, measured at 460 nm and record the absorbance.			

**VI. CALCULATION**

**Unit Definition:** One unit of GOD is defined as the enzyme that generates 1  $\mu\text{mol}$   $\text{H}_2\text{O}_2$  per minute.

1. According to the protein concentration of sample

$$\begin{aligned}\text{GOD (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times C_{\text{Protein}}) / T \\ &= 2.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{GOD (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 2.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned}\text{GOD (U}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 2.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned}\text{GOD (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}}/T \\ &= 2.5 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

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

$C_{\text{Standard}}$ : the concentration of Standard, 5 mmol/L = 5  $\mu\text{mol/ml}$ ;

W: the weight of sample, g;

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

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

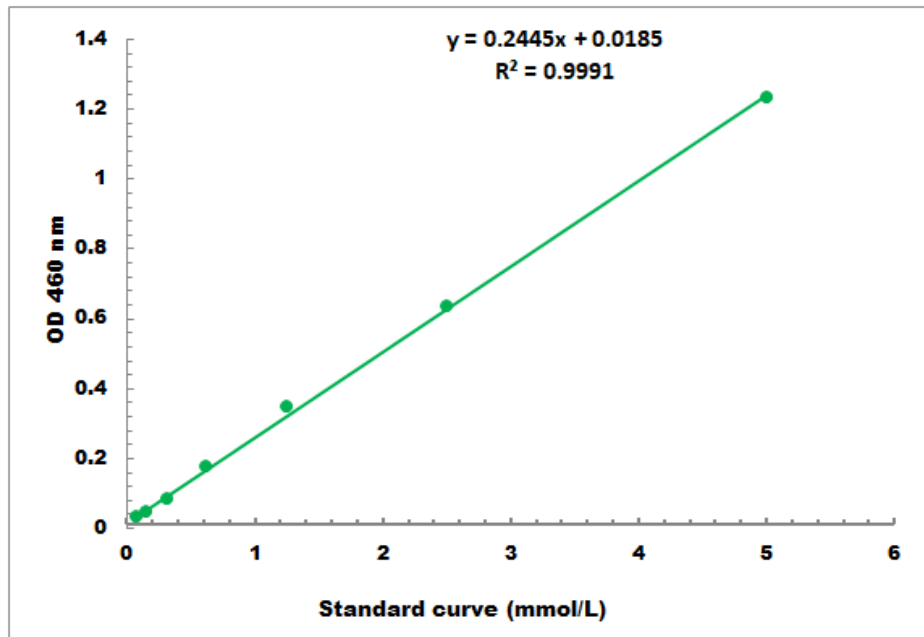
$V_{\text{Sample}}$ : the volume of sample, 0.02 ml;

$V_{\text{Assay}}$ : 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: 0.05 mmol/L - 5 mmol/L