# Glucose Oxidase Microplate Assay Kit

# User Manual

## Catalog # CAK1057

(Version 1.2E)

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 |
| Positive Control | Powder x 1 | -20 °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.

**Positive Control**: add 1 ml distilled water to dissolve before use,, then add 0.3 ml into 0.7 ml distilled water, mix.

## 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 $5 \times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 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 | Control | Standard | Blank | Positive Control |
|---|---|---|---|---|---|
| Dye Reagent | 10 µl | 10 µl | 10 µl | 10 µl | 10 µl |
| Substrate | 150 µl | 150 µl | -- | -- | 150 µl |
| Enzyme | 20 µl | 20 µl | 20 µl | 20 µl | 20 µl |
| Assay Buffer | -- | -- | 150 µl | 150 µl | -- |
| Mix. | | | | | |
| Sample | 20 µl | -- | -- | -- | -- |
| Standard | -- | -- | 20 µl | -- | -- |
| Positive Control | -- | -- | -- | -- | 20 µl |
| Distilled water | -- | 20 µl | -- | 20 µl | -- |
| 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.

3) Reagents must be added step by step, can not be mixed and added together.

## VI. CALCULATION

**Unit Definition:** One unit of GOD is defined as the enzyme that generates 1 μmol H2O2 per minute.

1. According to the protein concentration of sample

$$GOD\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 1.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$GOD\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 1.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$GOD\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 1.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$GOD\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 1.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the concentration of Standard, 3 mmol/L = 3 μmol/ml;

W: the weight of sample, g;

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

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

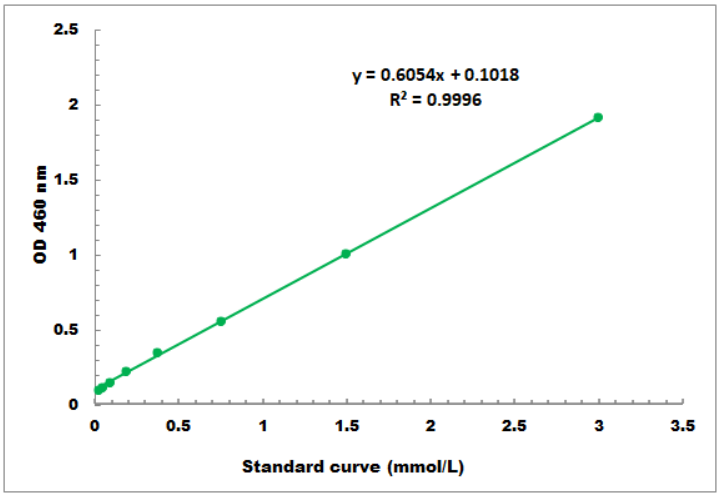
$V_{Sample}$: the volume of sample, 0.02 ml;

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

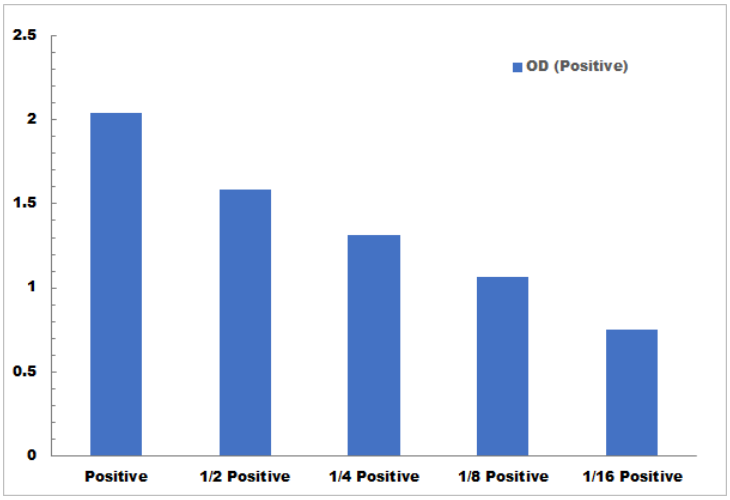
T: the reaction time, 2 minutes.

## 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




Positive Control reaction in 96-well plate assay with decreasing the concentration

## VIII. TECHNICAL SUPPORT

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

## IX. NOTES