Glucose Oxidase Microplate Assay Kit

Cat #: orb390763 (manual)

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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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.

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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.

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

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%, 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.

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.

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, $N \times 10^4$;

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

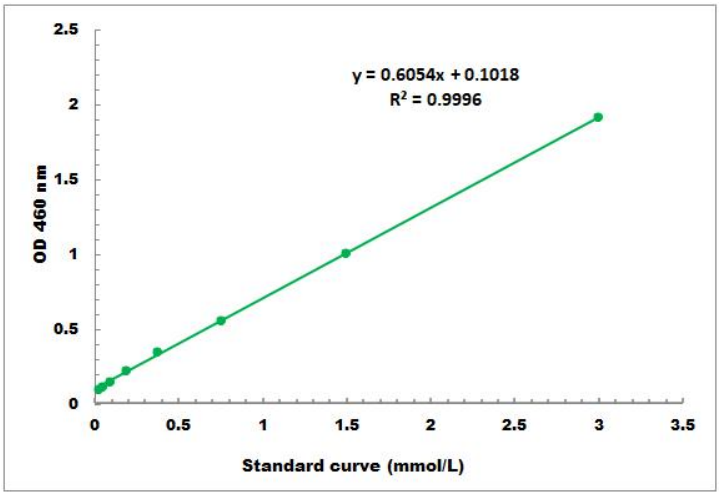
V_{Sample}: the volume of sample, 0.02 ml;

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

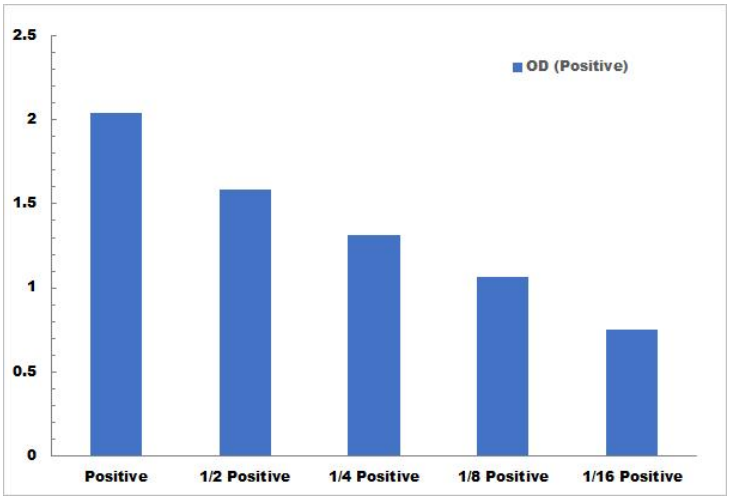
T: the reaction time, 2 minutes.

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

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