Glucose Oxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1057

(Version 1.3E)

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

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

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.

Glucose Oxidase ActivityColorimetric 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	30mlx 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	Powderx 1	-20 °C
Standard (3 mmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 3/1.5/0.75/0.375/0.188/0.094/0.047mmol/L.

Dye Reagent: Briefly centrifuge prior to opening. Add 1ml Dye Reagent Diluent to dissolve before use.Keep in dark and store at 4 °C for 1-2 months or -20°C for 3-6months after reconstitution.

Substrate: Add 15 ml Assay Buffer to dissolve before use.Store at 4 °C for 1 month or -20°C for 3-6 months after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Add 1ml Assay Buffer to dissolve before use.Store at -80°C for 1 month after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml distilled waterto dissolve before use,then add 0.3 ml into 0.7 ml distilled water, mix.Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Warm theDye Reagent,Substrate, Enzymeto room temperature before use.

Add following reagents intothe 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 recordthe absorbance.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of GOD is defined asthe enzyme generates1μmolH2O2 per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Control}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity}=\frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Control})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, mmol/L= μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

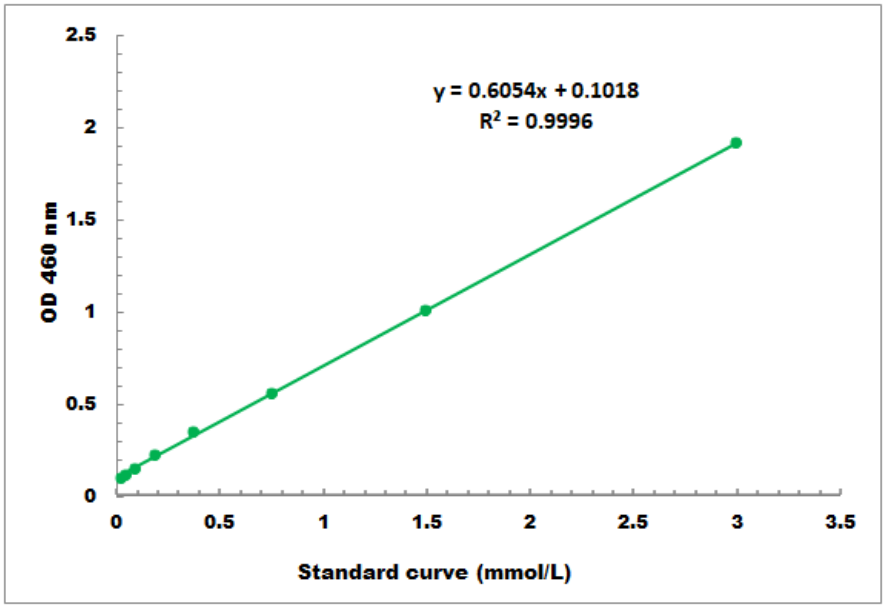
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

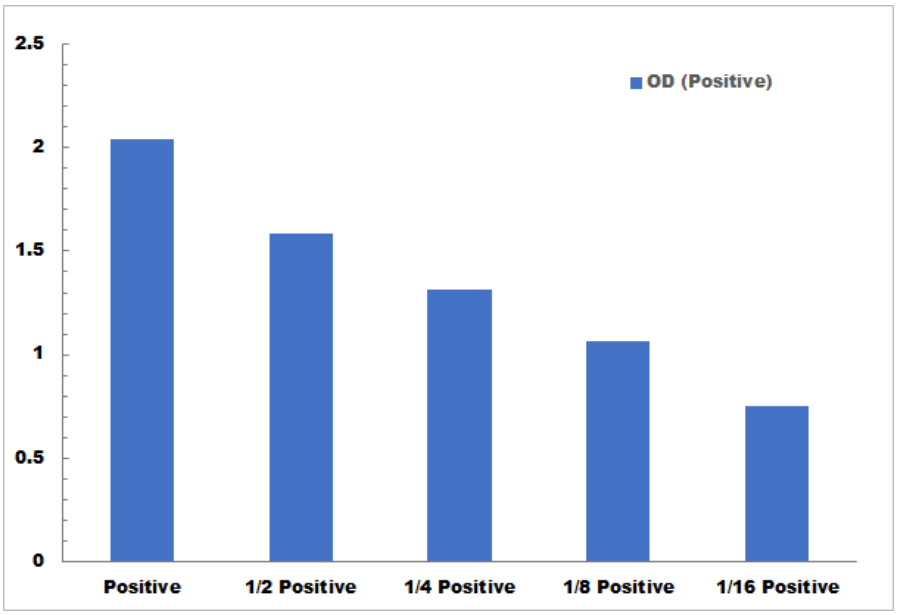
T: the reaction time, minute

VIII. 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