CheKine™ Micro Glucose Oxidase Activity (GOD) Assay Kit

Cat #: KTB1310 Size: 96 T/96 S 480 T/480 S

	Micro Glucose Oxidase Activity (GOD) Assay Kit		
REF	**Cat #:** KTB1310	LOT	**Lot #:** Refer to product label
	Detection range: 1-40 μM		**Sensitivity:** 1 μM
	Applicable samples: Serum, Plasma, Animal Tissues, Cells, Bacteria, Biological Fluids such as Urine		
	Storage: Stored at -20℃ for 12 months, protected from light		

Note: The detection range and sensitivity here refer to H_2O_2 standard, which need to be converted to GOD activity based on sample conditions.

Assay Principle

The glucose oxidase (GOD) (EC 1.1.3.4) is a dimeric enzyme that catalyzes oxidation of beta-D-glucose into hydrogen peroxide and D-glucono-1,5-lactone, which is hydrolyzed to gluconic acid. This enzyme is produced by certain species of insects, fungi, and bacteria. Glucose oxidase is widely used for the determination of glucose in body fluids and in removing residual glucose and oxygen from beverages, food and other agricultural products. Furthermore, Glucose oxidase is commonly used in biosensors to detect glucose. CheKine™ Micro Glucose Oxidase Activity (GOD) Assay Kit provides a simple and easy colorimetric assay for the study of Glucose Oxidase activity in a variety of biological samples such as Serum, Plasma, Animal Tissues, Cells, Bacteria, Biological Fluids such as Urine. The principle is that 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 chromogen in an acid condition, produce a product that can be measured at OD580 nm. Therefore, the glucose oxidase activity present in the sample is proportional to the signal obtained.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	96 T	480 T	
Assay Buffer (20×)	10 mL	50 mL	4℃
Glucose (0.2 M)	7 mL	35 mL	-20℃
H_2O_2 Standard (0.88 M)	100 μL	100 μL	-20℃, protect from light
Chromogen	5.6 mL	28 mL	-20℃, protect from light
Glucose Oxidase (Control)	100 μL	100 μL	-20℃, protect from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 580 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, ice maker, refrigerated centrifuge
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

1×Assay Buffer: Equilibrate to room temperature before use. Make a 1:20 dilution of the concentrated Assay Buffer (20×) with deionized water. 1×Assay Buffer should be used to dilute the H_2O_2 standards and samples prior to assay. 1×Assay Buffer is stored at 4℃ for at least two months.

Glucose (0.2 M): Ready to use as supplied. Equilibrate to room temperature before use. Please aliquot the unused reagents and store at -20℃.

H_2O_2 Standard (0.88 M): Ready to use as supplied. Equilibrate to room temperature before use, protected from light during the assay. Please aliquot the unused reagents and store at -20℃, protect from light.

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use, protected from light during the assay. Please aliquot the unused reagents and store at -20℃, protect from light.

Note: Chromogen is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Working Glucose Oxidase (Control): The vial contains Glucose Oxidase from aspergillus niger, which is used as a positive control. Take 2 μL Glucose Oxidase (Control) and dilute with 998 μL 1×Assay Buffer as the first step. After mixing, 1 μL of first diluted solution was diluted in 399 μL 1×Assay Buffer to obtain Working Glucose Oxidase (Control). During the whole experiment, place on ice from light. Please aliquot the undiluted reagents and store at -20℃, protect from light.

Standard preparation: Prepare 10 mM of H_2O_2 Standard by diluting 10 μL 0.88 M H_2O_2 Standard into 870 μL 1×Assay Buffer. Prepare 40 μM of H_2O_2 Standard by diluting 4 μL 10 mM H_2O_2 Standard into 996 μL 1×Assay Buffer. Using 40 μM standard, prepare standard curve dilution as described in the table:

Standard	Volume of 40 μM Standard (μL)	1×Assay Buffer (μL)	Final Concentration (μM)
1	200	0	40
2	150	50	30
3	100	100	20
4	50	150	10
5	25	175	5
6	10	190	2
7	5	195	1

Note: Always prepare fresh standards for every use. Diluted standard solution is unstable and must be used within 4 h.

Sample Preparation

1. Animal Tissue samples: Perfuse tissue with cold PBS to remove any red blood cells. Homogenize tissue at 1 mL/0.1 g in cold 1×Assay Buffer. Centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, keep on ice during the assay.
2. Cells and Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL cold 1×Assay Buffer to ultrasonically disrupt the cells or bacteria in ice bath 5 min

(power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 5 min at 4℃. Use supernatant for assay, keep on ice during the assay.

3. Plasma, Serum, Urine (and other biological fluids): Tested directly. However, to find the optimal values and ensure your readings will fall within the standard values, it is recommended that the sample be diluted in 1×Assay Buffer into different concentrations to test.

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ for one month. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or vsible spectrophotometer for more than 30 min, and adjust the wavelength to 580 nm, Visible spectrophotometer was returned to zero with deionized water.

2. Add the following reagents to the EP tube:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
1×Assay Buffer	50	0	0	0
Stds	0	50	0	0
Sample	0	0	50	0
Working Glucose Oxidase (Control)	0	0	0	50
Glucose (0.2 M)	50	50	50	50
Cover tightly and mix well. Incubate for 20 min at 37℃, add 60 μL of the above mixture into a 96-well plate or microglass cuvette, and quickly add 40 μL Chromogen to each well (a multi-channel pipette is recommended). Tap the plate and mix thoroughly, and incubating at 37℃ for 10 min protect from light, measure the absorbance A at 580 nm, recorded as A_{Blank}, $A_{Standard}$, A_{Test} and $A_{Control}$ respectively. Finally, calculate $\Delta A_{Standard}=A_{Standard}-A_{Blank}$, $\Delta A_{Test}=A_{Test}-A_{Blank}$.				

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 1-2 samples. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted, the calculated result multiplied by the dilution factor. If A_{Test} is less than 0.005, the sample size can be increased appropriately increased.

Data Analysis

1.Drawing of standard curve

With the concentration of the H_2O_2 Standard (μM) as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (1 μM=1 nmol/mL). It's the H_2O_2 content.

2.Calculate the Activity of GOD in sample

(1) Calculated by fresh weight of samples

Unit Definition: 1 nmol H_2O_2 produced per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

GOD (U/g) = y÷W÷T×n**=0.05×y÷W×n**

(2) Calculated by bacteria or cell number

Unit Definition: 1 nmol H_2O_2 produced per min in 10^4 bacteria or cell reaction system is defined as a unit of enzyme activity.

GOD (U/10^4) = y÷number of bacteria or cells÷T×n=y÷500÷T×n **=0.0001×y×n**

(3) Calculated by volume of liquid sample

Unit Definition: 1 nmol H_2O_2 produced per min in 1 mL Liquid sample reaction system is defined as a unit of enzyme activity.

GOD (U/mL) = y÷V_{Sample}÷T×n**=y×n**

(4) By protein concentration

Unit Definition: 1 nmol H_2O_2 produced per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

GOD (U/mg prot) = y÷Cpr÷T×n**=0.05×y÷Cpr×n**

Where: W: sample weight, g; T: reaction time, 20 min; n: dilution factor; 500: Total number of bacteria or cells; V_{Sample}: sample volume added, 0.05 mL; Cpr: sample protein concentration, mg/mL.

Typical Data

Typical standard curve

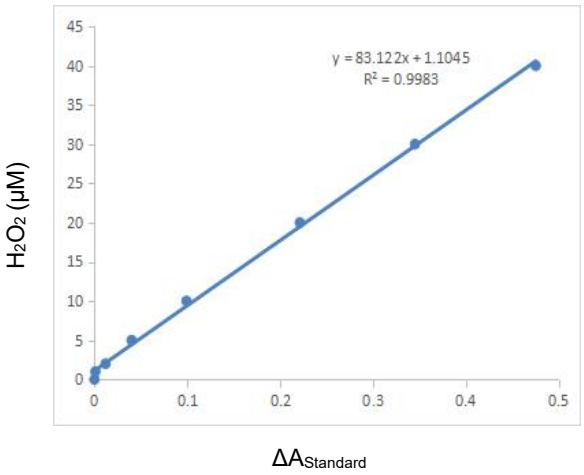

Figure 1. Standard curve of GOD in 96-well plate assay–data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1041	CheKine™ Micro Hydrogen Peroxide (H_2O_2) Assay Kit
KTB1210	CheKine™ Micro Superoxide Anion Assay Kit
KTB1220	CheKine™ Micro Diamine Oxidase (DAO) Activity Assay Kit
KTB1200	CheKine™ Micro Protein Carbonyl Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.