



TECHNICAL MANUAL

Glucose Oxidase (GOD) Activity Assay Kit

- **SKU CODE:** MAES0248
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

-  (img-0.jpeg)

2. Intended use

This kit can be used to measure glucose oxidase (GOD) activity in samples.

3. Detection principle

Glucose oxidase is an important industrial enzyme in the food industry, widely used in wine, beer, juice, milk powder, and other food applications for deoxygenation, flour improvement, and food browning prevention. It is also extensively utilized in food rapid detection and biosensors. Microorganisms are the main source of GOD production due to their rapid growth, reproduction, and wide availability, with the primary production strains being *Aspergillus niger* and *Penicillium*.

Glucose oxidase catalyzes the oxidation of glucose to produce hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidizes chromogenic substrates to form colored compounds. The optical density (OD) value is measured at 550 nm, and glucose oxidase (GOD) activity can be calculated indirectly.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	15 mL x 1 vial	30 mL x 1 vial	-20°C, 12 months
Reagent 2	Enzyme Reagent	Powder x 1 vial	Powder x 2 vials	-20°C, 12 months, protect from light
Reagent 3	Chromogenic Agent	1.6 mL x 1 vial	1.6 mL x 2 vials	-20°C, 12 months, protect from light
Reagent 4	1 mmol/L Standard	1.6 mL x 1 vial	1.6 mL x 2 vials	-20°C, 12 months, protect from light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Pipettor, Water bath, Centrifuge, Microplate reader (540-560 nm)

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to ensure sufficient reagent recovery.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 0.2 mL double distilled water. Store aliquots at -20°C for up to 1 week. Avoid repeated freeze/thaw cycles.
3. **Preparation of reaction working solution:** For each well, prepare 200 μ L of reaction working solution by mixing 196 μ L of buffer solution with 4 μ L of enzyme working solution. The reaction working solution should be prepared immediately before use. Keep on ice and use within 4 hours.
4. **Preparation of standard curve:** Always prepare fresh standards. Discard working standard dilutions after use. Dilute the 1 mmol/L standard with double distilled water to create a serial concentration series. The recommended dilution gradient is: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1 mmol/L.

7. Sample preparation

1. **Sample preparation:** Use normal saline (0.9% NaCl) as the homogenate medium. Centrifuge at 10,000 x g for 10 minutes to remove insoluble material. Collect the supernatant and keep on ice for detection. Meanwhile, determine the protein concentration of the supernatant (MAES0177).
2. **Dilution of sample:** The diluent is normal saline (0.9% NaCl). For dilution of other sample types, perform a pretest to confirm the appropriate dilution factor.

8. Operating steps

1. Standard wells: Add 10 μ L of standard solution with different concentrations to the wells. Sample wells: Add 10 μ L of sample to the wells.
2. Add 200 μ L of reaction working solution to each well.
3. Add 20 μ L of chromogenic agent to each well.

4. Mix thoroughly for 3 seconds with microplate reader. Measure the OD value of each well at 550 nm, recorded as A1.
5. Incubate at 37°C for 20 minutes.
6. Mix thoroughly for 3 seconds with microplate reader. Measure the OD value of each well at 550 nm, recorded as A2. Calculate $\Delta A = A2 - A1$. (Note: There is no change in OD value of standard wells; plot the standard curve using the A2 OD values of standards.)

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.
2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This gives the absolute OD value.
3. Plot the standard curve using the absolute OD value of standards as the y-axis and the corresponding concentration as the x-axis. Create the standard curve ($y = ax + b$) using graphing software or Excel.

The sample:

Definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 μmol of hydrogen peroxide per gram of sample protein per minute at 37°C.

$$\text{GOD activity (U/g}_{\text{prot}}) = (\Delta A_{550} - b) \div a \div T \times 1000 \div C_{\text{pr}} \times f$$

[Note]

ΔA_{550} : $\Delta A_{\text{sample}} (A_2 - A_1)$

T: Reaction time (20 min)

1000: 1 mmol/L = 1000 $\mu\text{mol/L}$

C_{pr} : Concentration of protein in sample ($\text{g}_{\text{prot}}/\text{L}$)

f: Dilution factor of sample before test

10. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.03	0.07	0.09
%CV	3.4	3.2	2.4

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.03	0.07	0.09
%CV	6.8	7.1	7.1

Recovery

Three samples of high, medium, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 101%.

Recovery

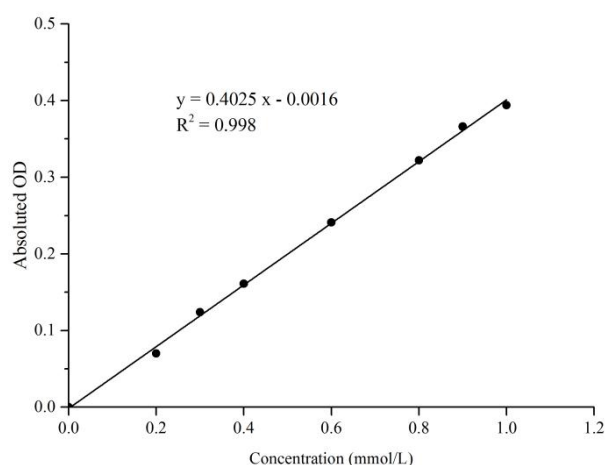
Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.28	0.54
Observed Conc. (mmol/L)	0.3	0.5
Recovery rate (%)	101	99

Sensitivity

The analytical sensitivity of the assay is 0.006 U/L activity. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to actual assay performance conditions (e.g., operator, pipetting technique, or temperature effects), the standard curve and data provided below are for reference only:



Standard curve

Concentration (mmol/L)	0	0.2	0.3	0.4	0.6	0.8	0.9	1
OD value	0.041	0.112	0.166	0.202	0.279	0.362	0.41	0.432
	0.042	0.111	0.165	0.203	0.286	0.365	0.405	0.439
Average OD	0.042	0.112	0.166	0.203	0.283	0.364	0.408	0.436
Absolute OD	0	0.07	0.124	0.161	0.241	0.322	0.366	0.394

11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing experiments. Follow the instructions strictly throughout the procedure.
3. Protective measures must be taken by wearing a lab coat and latex gloves.
4. If the concentration of the substance is not within the detection range, perform additional dilution or concentration steps on the sample.
5. It is recommended to perform a pretest if your sample type is not listed in the instruction manual.
6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.



Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.