（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K520-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（540-560 nm）

Detection range：0.006–0.1 U/L

Elabscience® Glucose Oxidase (GOD) Activity Assay Kit

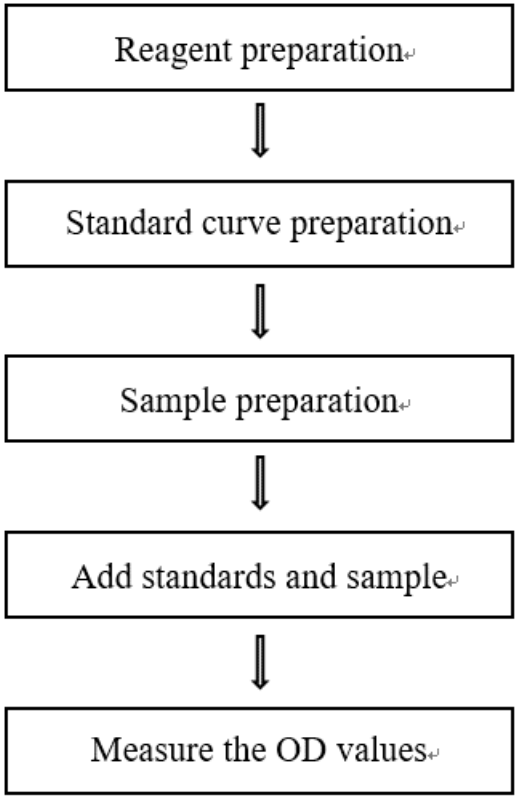
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

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

Detection principle

Glucose oxidase is an important industrial enzyme in the food industry, widely used in wine, beer, juice, milk powder and other food deoxygenation, flour improvement, food browning prevention and other aspects, in food rapid detection and biosensors are also widely used. Microorganisms are the main source of GOD production with rapid growth and reproduction and wide sources, and the main production strains are aspergillus niger and penicillium.

Glucose oxidase can catalyze the oxidation of glucose to produce hydrogen peroxide. In the presence of peroxidase, hydrogen oxidizes pigment sources to form colored substances. Measure the OD value at 550 nm and glucose oxidase (GOD) activity can be calculated indirectly.

Kit components & storage

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

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 a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of enzyme working solution：

Dissolve a vial of enzyme reagent with 0.2 mL double distilled water.

Aliquoted storage at -20 ℃ for 1 week, and avoid repeated freeze/thaw cycles is advised.

③ The preparation of reaction working solution：

For each well, prepare 200 μL of reaction working solution (mix well 196 μL of buffer solution and 4 μL of enzyme working solution). The reaction working solution should be prepared on spot. Keep it on the ice for use, and use up within 4 h

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**0.9**	**1**
1 mmol/L Standard Solution (μL)	0	20	30	40	60	80	90	100
Double distilled water (μL)	100	80	70	60	40	20	10	0

Sample preparation

① Sample preparation

Sample:

The homogenate medium is normal saline is normal saline (0.9% NaCl). Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor

Operating steps

① Standard well: Take 10 μL of standard solution with different concentration to the wells.

Sample well: Take 10 μL of sample to the wells.

② Add 200 μL of reaction working solution into the each well.

③ Add 20 μL of chromogenic agent into each well.

④ Mix fully for 3 s with microplate reader. Measure the OD value of each well with microplate reader at 550 nm, recorded as A_1.

⑤ Incubate at 37℃ for 20 min.

⑥ Mix fully for 3 s with microplate reader. Measure the OD value of each well with microplate reader at 550 nm, recorded as A_2, $\triangle A=A_2-A_1$. (Note: There is no change in OD value of standard well, plot the standard curve with the OD value of A_2 (standard)).

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Definition: the enzyme amount of 1 μmol of hydrogen generated by 1 g sample protein per minute at 37℃ is defined as 1 unit.

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

[Note]

ΔA_{550}: $\Delta_{Sample}(A_2-A_1)$

T: Reaction time (20 min)

1000: 1 mmol/L=1000 μmol/L

C_{pr}: Concentration of protein in sample (gprot/L)

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.28	0.54	0.83
Observed Conc. (mmol/L)	0.3	0.5	0.9
Recovery rate (%)	101	99	103

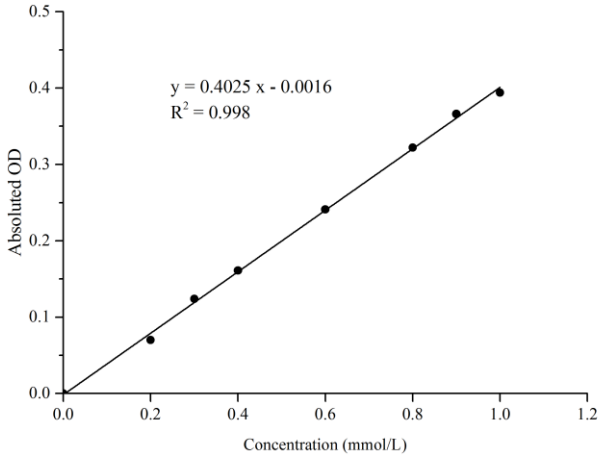
Sensitivity

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.

2. Standard curve:

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

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
Absoluted OD	0	0.07	0.124	0.161	0.241	0.322	0.366	0.394



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.