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

Catalog No：E-BC-F094

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

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.1-17.4 mU/mL

Elabscience® Glutamate Oxidase (GLOD) Activity Fluorometric 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.

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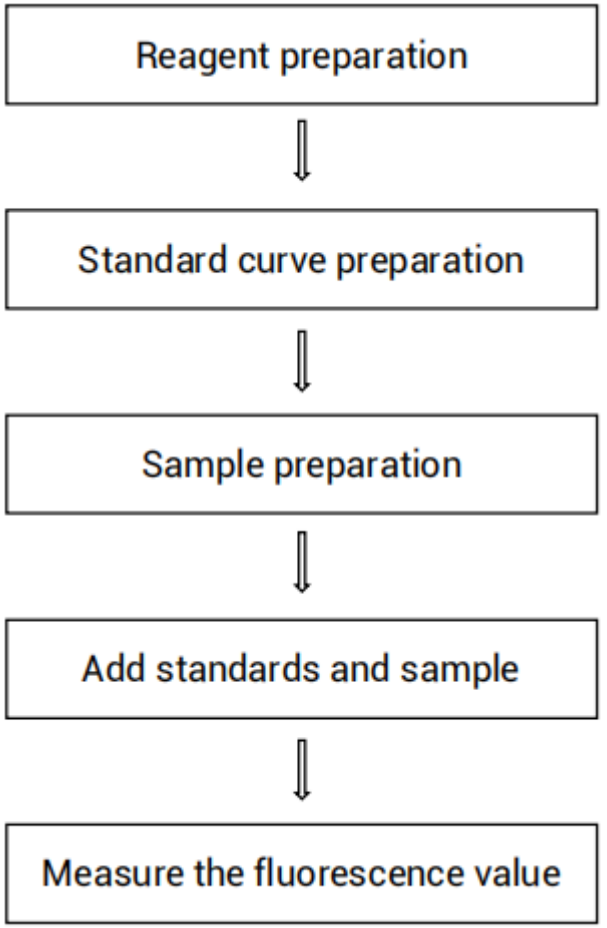
Assay summary



Intended use

This kit can be used to measure activity of purified glutamate oxidase (GLOD).

Detection principle

L-glutamate oxidase (GLOD) is an L-amino acid oxidase with flavin adenine dinucleotide (FAD) as the coenzyme. However, due to its own presence of a non-covalently bound FAD, Therefore, the catalytic reaction can be completed without the addition of exogenous cofactor FAD, and L-glutamic acid can be deaminated to generate α-ketoglutaric acid, ammonia and hydrogen peroxide. L-glutamate oxidase has high specificity and affinity for reaction substrates, mild reaction conditions and high catalytic efficiency, and is widely used in the food, industrial fermentation and pharmaceutical industries.

The detection principle of this kit: Glutamate oxidase oxidizes the substrate, and the resulting substance reacts with the chromogenic reagent to produce fluorescence. The fluorescence value is detected at the excitation wavelength of 535 nm and the emission wavelength of 587 nm. The enzyme activity is calculated based on the fluorescence value of the standard.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	8 mL × 1 vial	16 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Chromogenic Agent	0.25 mL × 1 vial	0.5 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	1 mmol/L Standard Solution	0.25 mL × 1 vial	0.5 mL ×1 vial	-20°C, 12 months shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator

Reagents:

Normal saline (0.9%NaCl)

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of working solution:

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 200 μL of working solution (mix well 194 μL of buffer solution and 6 μL of chromogenic agent). The working solution should be prepared on spot protected from light and used up within 8 h.

③ The preparation of 10 μmol/L standard solution:

Before testing, please prepare sufficient 10 μmol/L standard solution. For example, prepare 1000 μL of 10 μmol/L standard solution (mix well 10 μL of 1 mmol/L standard solution and 990 μL of double distilled water). The standard solution should be prepared on spot protected from light and used up within 8 h.

④ The preparation of standard curve:

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

Dilute 10 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 3, 4, 6, 7, 8, 10 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**2**	**3**	**4**	**6**	**7**	**8**	**10**
10 μmol/L Standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① **Sample preparation**

Purified enzyme: detect directly.

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: add 20 μL of standard solution with different concentrations into the wells.

Sample well: add 20 μL of sample into the wells.

② Add 140 μL of working solution into each well.

③ Mix fully with fluorescence microplate for 5s. Measure the fluorescence at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, as F_1. Incubate at 37°C for 10 min protected from light. Measure the fluorescence at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, as F_2. The standard curve is fitted to the standard well in F_2 value.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean fluorescence value of the blank (Standard #①) from all standard readings. This is the absoluted fluorescence value.
3. Plot the standard curve by using absoluted fluorescence 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:

Samples:

Definition: The amount of enzyme in 1 g protein per 1 min that produce 1 μmol of production at 37°C is defined as 1 unit.

$$\text{GLOD activity (mU/mgprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

[Note]

ΔF: The absolute fluorescence value of sample well ($\Delta F = F_2 - F_1$).

T: Reaction time, 10 min.

f: Dilution factor of sample before tested.

C_{pr}: The concentration of protein in sample, mgprot/mL.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three GLOD 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 (mU/mL)	5.00	10.00	15.00
%CV	3.3	5.0	4.9

Inter-assay Precision

Three GLOD samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mU/mL)	5.00	10.00	15.00
%CV	4.6	7.3	10.0

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 100.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc(mU/mL)	5.00	10.00	15.00
Observed Conc(mU/mL)	5.0	9.6	15.8
Recovery rate (%)	100	96	105

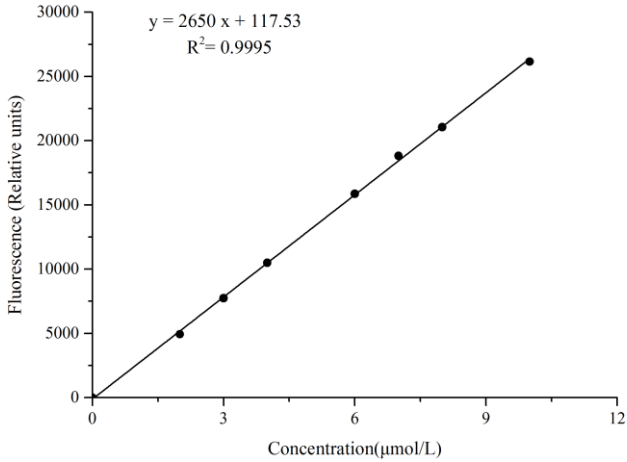
Sensitivity

The analytical sensitivity of the assay is 0.1 mU/mL. 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.

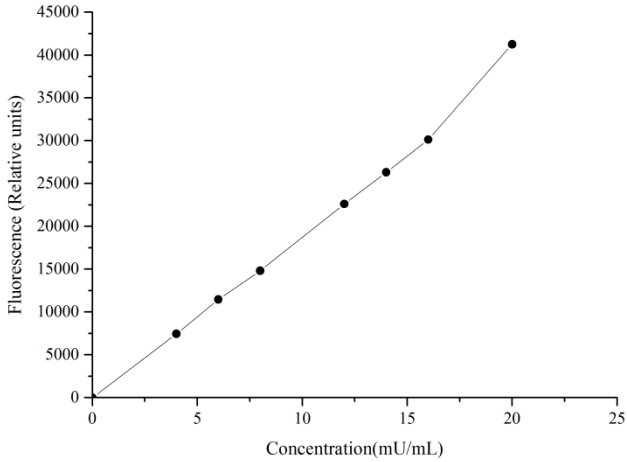
2. Standard curve:

As the fluorescence 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 (μmol/L)	0	2	3	4	6	7	8	10
F_2 value	1063	6097	8853	11711	16883	19723	22193	27141
	1049	5878	8728	11395	16954	20021	22042	27283
Average F_2 value	1056	5988	8791	11553	16919	19872	22118	27212
Absoluted F_2 value	0	4932	7735	10497	15863	18816	21062	26156



3. Dilution curve of GLOD standard enzyme



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.