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

Catalog No：E-BC-K524-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（450-470 nm）

Detection range：7.39-134.15 U/L

Elabscience® Diamine Oxidase (DAO) 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.

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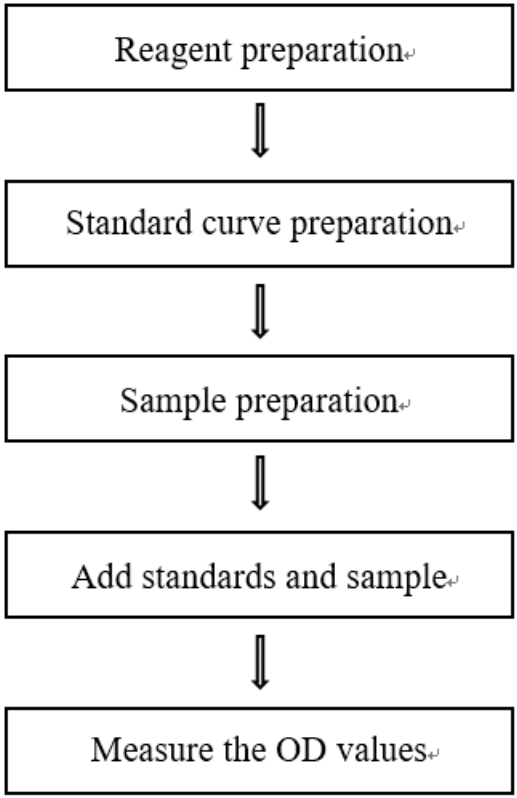
Assay summary



Intended use

This kit can be used to measure diamine oxidase (DAO) activity in serum, plasma, urine, animal tissue and cell samples.

Detection principle

Diamine oxidase (DAO,E.C.1.4.3.6) exists widely in animal tissues (intestinal mucosa, lung, liver, kidney, etc.), serum, plasma and cells. It is a highly active intracellular enzyme in the upper villi of human and mammalian small intestinal mucosa. It can regulate the intracellular ion balance, affect the conduction pathway and promote cell repair. The detection principle of this kit is that diamine oxidase can catalyse amine substances to produce hydrogen peroxide, and hydrogen peroxide can react with the chromogenic substance to produce chromogenic substance, which has a characteristic absorption peak at 460 nm. The activity of diamine oxidase can be calculated by measuring the change rate of absorbance per unit time..

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	60 mL ×1 vial	2-8℃, 12 months
Reagent 2	Substrate	1.5 mL ×2 vials	2-8℃, 12 months shading light
Reagent 3	Enzyme Reagent	Powder ×1 vial	2-8℃, 12 months shading light
Reagent 4	Chromogenic Agent	Powder ×1 vial	2-8℃, 12 months shading light
Reagent 5	Stop Agent	10 mL ×1 vial	2-8℃, 12 months
Reagent 6	100 mmol/L Standard	1 mL ×1 vial	2-8℃, 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:

Centrifuge, Incubator, Microplate reader (450-470 nm, optimum wavelength: 460 nm).

Reagent preparation

① Preserve the enzyme reagent on the ice, and equilibrate other reagents to room temperature before use.

② The preparation of enzyme stocking solution:
Dissolve a vial of enzyme reagent with 1.5 mL double distilled water and mix well. Store at 2-8 ℃ for 7 days protected from light.

③ The preparation of enzyme working solution:
For each well, prepare 100 μL of enzyme working solution (mix well 10 μL of enzyme stocking solution and 90 μL of buffer solution). The enzyme working solution should be prepared on spot and keep it on ice protected from light for detection.

④ The preparation of chromogenic working solution:
Dissolve a vial of chromogenic agent with 1.9 mL double distilled water. Store at 2-8 ℃ for 7 days protected from light.

⑤ The preparation of 5 mmol/L standard:
Dilute 15 μL of 100 mmol/L standard with 285 μL of double distilled water. Prepare the fresh needed amount before use, and store at 2-8 ℃ for 7 days

protected from light.

⑥ The preparation of standard curve：

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

Dilute 5 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.0, 0.2, 0.5, 0.7, 1.0, 1.2, 1.5, 2.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.5**	**0.7**	**1.0**	**1.2**	**1.5**	**2.0**
5 mmol/L Standard Solution (μL)	0	8	20	28	40	48	60	80
Double distilled water (μL)	200	192	180	172	160	152	140	120

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If the sample is turbidity, centrifuge at 2000 g for 10 min, then take the supernatant for detection. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cells:

① Harvest the number of cells needed for each assay (initial recommendation 2×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 2×10^6 cells in 200 μL buffer solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	2-4
Human plasma	1
Rat serum	1
Rat plasma	1
Mouse serum	1
Mouse plasma	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat heart tissue homogenate	1
Human urine	1
Hela Cells (1×10^6)	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① Prevent the formulation of bubbles when stop agent is transferred into the microplate.

② If the detection system is turbidity after adding stop agent, mix the reaction system.

③ If there is solid substance in stop agent, heat it at 60℃ for 5 min until clear.

Operating steps

① Standard well: Take 20 μL of standard solution with different concentrations to the corresponding wells.

Control well: Take 20 μL of sample to the corresponding wells.

Sample well: Take 20 μL of sample to the corresponding wells.

② Add 20 μL of substrate and 100 μL of enzyme working solution to each well.

③ Add 30 μL of double distilled water into the control wells.

Add 30 μL of chromogenic working solution into the standard wells and sample wells.

④ Mix fully with microplate reader and incubate at 37℃ for 30 min.

⑤ Add 50 μL of stop agent into each well (Please take and beat slowly to avoid large bubbles).

⑥ Mix fully with microplate reader and incubate at 37℃ for 5 min.

⑦ Measure the OD value of each well with microplate reader at 460 nm (Break the bubbles before measurement if there are some bubbles).

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:

1. Serum (plasma) sample:

Definition: The amount of enzyme in 1 L of serum (plasma) that hydrolysis the substrate to produce 1μmol substance at 37℃ for 1 min is defined as 1 unit.

$$\text{DAO activity (U/L)} = \frac{\Delta A_{460} - b}{a} \div T \times f \times 1000^*$$

2. Tissue sample and cells sample:

Definition: The amount of enzyme in 1 g of tissue protein that hydrolysis the substrate to produce 1μmol substance at 37℃ for 1 min is defined as 1 unit.

$$\text{DAO activity (U/gprot)} = \frac{\Delta A_{460} - b}{a} \div C_{pr} \div T \times f \times 1000^*$$

[Note]

ΔA_{460}: $OD_{Sample} - OD_{Control}$.

T: The time of incubation reaction, 30 min.

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

1000*: 1 mmol/L =1000 μmol/L.

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)	18.4	62.5	112.0
%CV	5.6	5.2	4.2

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)	18.4	62.5	112.0
%CV	6.4	7.2	7.4

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 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc.（mmol/L）	0.35	0.94	1.3
Observed Conc.（mmol/L）	0.4	1.0	1.4
Recovery rate (%)	101	106	108

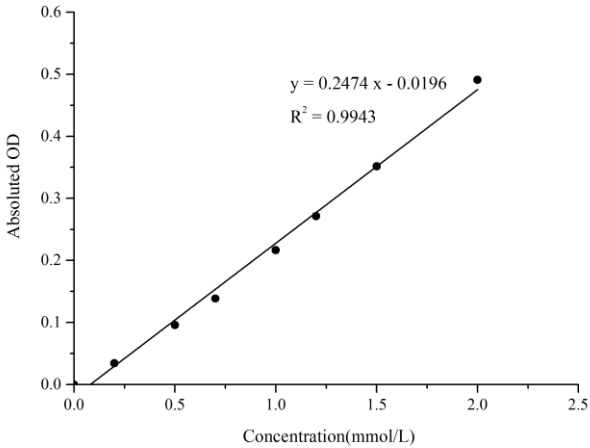
Sensitivity

The analytical sensitivity of the assay is 7.39 U/L. 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**	**0.5**	**0.7**	**1.0**	**1.2**	**1.5**	**2.0**
OD value	0.065	0.098	0.161	0.198	0.280	0.336	0.413	0.556
	0.064	0.100	0.160	0.208	0.282	0.336	0.419	0.555
Average OD	0.065	0.099	0.161	0.203	0.281	0.336	0.416	0.556
Absoluted OD	0.000	0.035	0.096	0.139	0.217	0.272	0.352	0.491



Appendix II Example Analysis

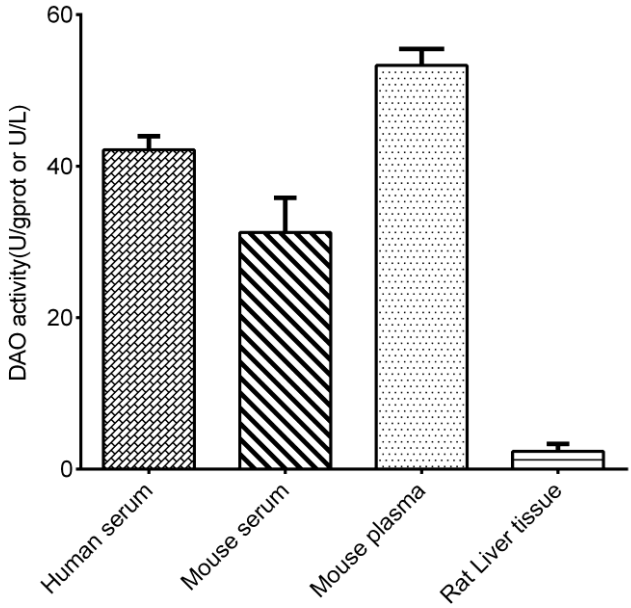
Example analysis:

For human serum, take 20 μL of human serum diluted for 2 times, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.2474 x - 0.0196, the average OD value of the control is 0.058, the average OD value of the sample is 0.194, and the calculation result is:

DAO activity (U/L) = (0.194 – 0.058 + 0.0196) ÷ 0.2474 ÷ 30 × 2 × 1000 = 41.93 U/L

Detect human serum (dilute for 2 times), mouse serum, mouse plasma and 10% rat liver tissue homogenate (the concentration of protein is 10.73gprot/L) according to the protocol, the result is as follows:



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.