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

Catalog No：E-BC-F093

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.003-2.67 U/L

Elabscience® Monoamine Oxidase (MAO) Activity Fluorometric Assay Kit

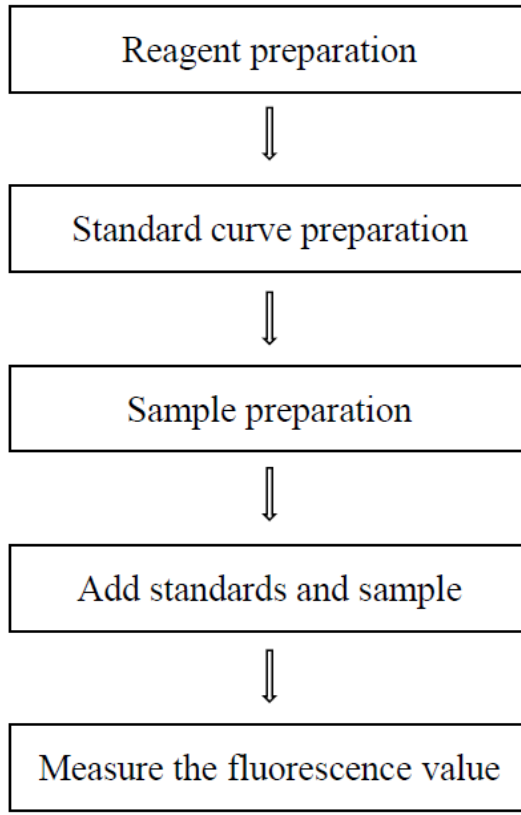
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure monoamine oxidase (MAO) activity in serum, plasma, animal tissue and cell samples, and can also specifically detect the activity of monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B).

Detection principle

Monoamine Oxidase (MAO) is widely present in mitochondria of liver, kidney, brain, small intestine and other organ cells, and mainly participates in metabolic inactivation of bioactive amine substances. MAO is divided into two subtypes, MAO-A and MAO-B, based on the binding specificity of substrates or inhibitors, cell distribution, and immune specificity. MAO can catalyze the corresponding substrate to produce H_2O_2, and H_2O_2 further forms a red complex with the fluorescent probe under the action of the enzyme. The change of the fluorescence value of the sample at the excitation wavelength of 535 nm and the emission wavelength of 587 nm in unit time can be measured, and the activity of MAO-A and MAO-B of the sample can be detected and calculated by inhibiting it with specific inhibitors.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	-20 ℃, 12 months
Reagent 2	Buffer Solution	40 mL × 1 vial	-20 ℃, 12 months shading light
Reagent 3	Inhibitor A	0.75 mL × 1 vial	-20 ℃, 12 months
Reagent 4	Inhibitor B	0.75 mL × 1 vial	-20 ℃, 12 months
Reagent 5	Enzyme Reagent	Powder × 2 vials	-20 ℃, 12 months shading light
Reagent 6	Accelerant	0.2 mL × 1 vial	-20 ℃, 12 months shading light
Reagent 7	Substrate	1.5 mL × 1 vial	-20 ℃, 12 months shading light
Reagent 8	Probe	3 mL × 1 vial	-20 ℃, 12 months shading light
Reagent 9	8 mmol/L Standard Solution	0.2 mL × 1 vial	-20 ℃, 12 months shading light
	Black 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:

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

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25 ℃ before use.

② The preparation of inhibitor A working solution：

For each well, prepare 40 μL of inhibitor A working solution (mix well 5 μL of inhibitor A and 35 μL of buffer solution). Store at 2-8 ℃ for one day protected from light or -20 ℃ for 1 month.

③ The preparation of inhibitor B working solution：

For each well, prepare 40 μL of inhibitor B working solution (mix well 5 μL of inhibitor B and 35 μL of buffer solution). Store at 2-8 ℃ for one day protected from light or -20 ℃ for 1 month.

④ The preparation of enzyme working solution：

Dissolve one vial of enzyme reagent with 1.25 mL of buffer solution, mix well to dissolve. Store at 2-8 ℃ for 7 days protected from light.

⑤ The preparation of measuring working solution：

Add 1.9 mL of buffer solution, 0.225 mL of enzyme working solution, 0.009 mL of accelerant and 0.126 mL of substrate into a 5 mL of EP tube, mix well. The measuring working solution should be prepared on spot and used up within 30 min.

⑥ The preparation of 80 μmol/L standard solution：

Before testing, please prepare sufficient 80 μmol/L standard solution. For example, prepare 1000 μL of 80 μmol/L standard solution (mix well 10 μL of 8 mmol/L standard solution and 990 μL of double distilled water). Store at 2-8 ℃ for 7 days protected from light.

⑦ The preparation of standard curve：

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

Dilute 80 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 16, 32, 40,

48, 56, 64, 80 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**16**	**32**	**40**	**48**	**56**	**64**	**80**
80 μmol/L Standard (μL)	0	40	80	100	120	140	160	200
Double distilled water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

Tissue sample:

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

② Wash tissue in normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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).

Cell (adherent or suspension) samples:

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

② Wash cells in 200 μL normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ 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	2-3
Mouse serum	2-4
10% Mouse liver tissue homogenate	2-4
10% Mouse kidney tissue homogenate	2-3
10% Mouse lung tissue homogenate	2-3
10% Mouse heart tissue homogenate	2-3
10% Mouse brain tissue homogenate	1
10% Shrimp tissue homogenate	1
1×10^6 CHO cells	1
1×10^6 293T cells	1

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

The key points of the assay

The measuring working solution should be prepared on spot and used up within 30 min to avoid the background value rising over time.

Operating steps

① Standard well: add 10 μL of standard with different concentrations into the well.

MAO-A sample well: add 10 μL of sample into the well.

MAO-B sample well: add 10 μL of sample into the well.

Control well: add 10 μL of sample into the well.

② Add 40 μL of buffer solution into standard wells and control wells.

Add 40 μL of inhibitor A working solution into MAO-A sample wells.

Add 40 μL of inhibitor B working solution into MAO-B sample wells.

③ Stand at 25 ℃ for 10 min.

④ Add 20 μL of probe into each well.

⑤ Add 140 μL of measuring working solution into each well.

⑥ Mix fully with fluorescence microplate reader for 5s. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, as F_1. Stand at 25 ℃ for 30 min. Measure the fluorescence intensity 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_1.)

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:

1. Serum and plasma samples:

Definition: the amount of enzyme in 1 L of serum or plasma that catalyze the substrate to produce 1 μmol product at 25 ℃ for 1 min is defined as 1 unit.

$$\text{MAO-A/MAO-B activity (U/L)} = ((\Delta F_{control} - \Delta F_{MAO\text{-}A/MAO\text{-}B}) - b) \div a \times f \div T$$

$$MAO_{total} \text{ activity} = \text{MAO-A} + \text{MAO-B}$$

2. Animal tissue and cell samples:

Definition: the amount of enzyme in 1 g of tissue or cell protein that catalyze the substrate to produce 1 μmol product at 25 ℃ for 1 min is defined as 1 unit.

$$\text{MAO-A/MAO-B activity (U/gprot)} = ((\Delta F_{control} - \Delta F_{MAO\text{-}A/MAO\text{-}B}) - b) \div a \div C_{pr} \times f \div T$$

$$MAO_{total} \text{ activity} = \text{MAO-A} + \text{MAO-B}$$

[Note]

$\Delta F_{control}$: $\Delta F_{control} = F_2 - F_1$.

$\Delta F_{MAO\text{-}A/MAO\text{-}B}$: $\Delta F_{MAO\text{-}A/MAO\text{-}B} = F_2 - F_1$

T: The time of incubation in the reaction, 30 min.

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

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse serum 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)	1.00	1.50	2.00
%CV	1.5	5.2	3.6

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	1.00	1.50	2.00
%CV	3.4	10.3	9.4

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 102%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/L)	1.0	1.5	2
Observed Conc.(U/L)	0.96	1.6	2.2
Recovery rate (%)	96	106	110

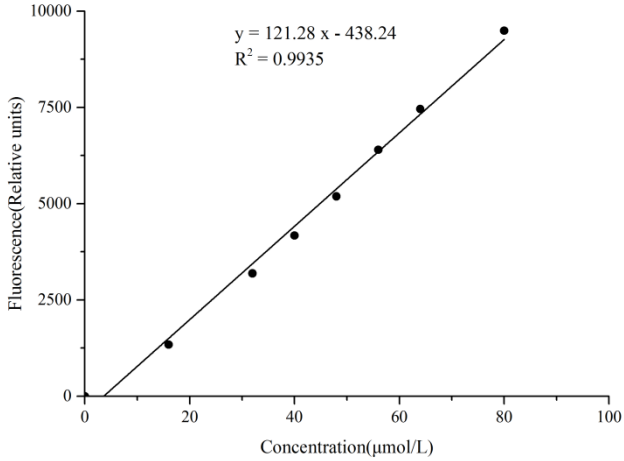
Sensitivity

The analytical sensitivity of the assay is 0.003 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 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	16	32	40	48	56	64	80
Fluorescence value	238	1517	3407	4505	5492	6598	7727	9626
	200	1603	3407	4277	5327	6638	7624	9802
Average fluorescence value	219	1560	3407	4391	5410	6618	7675	9714
Absoluted fluorescence value	0	1341	3189	4172	5191	6399	7457	9495



Appendix II Example Analysis

Example analysis:

Take 10 μL of human serum which dilute for 2 times and carry the assay according to the operation steps. The results are as follows:

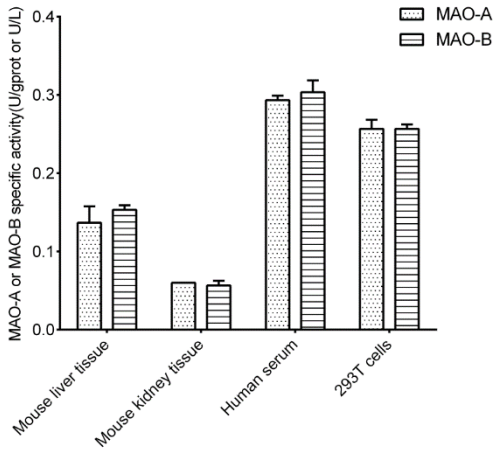
Standard curve: y = 121.28 x - 438.24, the average F_1 of the control well is 197, the average F_2 of the control well is 488, the average F_1 of the MAO-A sample well is 192, the average F_2 of the MAO-A sample well is 394, the average F_1 of the MAO-B sample well is 200, the average F_2 of the MAO-B sample well is 381, and the calculation result is:

$$\text{MAO-A activity (U/L)} = (488 - 197 - (394 - 192) + 438.24) \div 121.28 \times 2 \div 30 = 0.29 \text{ U/L}$$

$$\text{MAO-B activity (U/L)} = (488 - 197 - (381 - 200) + 438.24) \div 121.28 \times 2 \div 30 = 0.30 \text{ U/L}$$

$$\text{MAO}_{total} \text{ activity (U/L)} = 0.29 + 0.30 = 0.59 \text{ U/L}$$

Detect 10% mouse liver tissue homogenate (the concentration of protein is 7.90 gprot/L, dilute for 2 times), 10% mouse kidney tissue homogenate (the concentration of protein is 9.78 gprot/L, dilute for 2 times), human serum (dilute for 2 times) and 1×10^6 293T cells (the concentration of protein is 0.49 gprot/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.