



TECHNICAL MANUAL

Monoamine Oxidase (MAO) Activity Fluorometric Assay Kit

- **SKU CODE:** MAES0329
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure fluorescence values
- Calculate MAO activity

2. 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).

3. 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 H₂O₂, and H₂O₂ further forms a red complex with the fluorescent probe under the action of the enzyme. The change in fluorescence value of the sample at excitation wavelength of 535 nm and emission wavelength of 587 nm per 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.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	-20°C, 12 months
Reagent 2	Buffer Solution	40 mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Inhibitor A	0.75 mL × 1 vial	-20°C, 12 months

Item	Component	Size (96 T)	Storage
Reagent 4	Inhibitor B	0.75 mL × 1 vial	-20°C, 12 months
Reagent 5	Enzyme Reagent	Powder × 2 vials	-20°C, 12 months, shading light
Reagent 6	Accelerant	0.2 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	Substrate	1.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 8	Probe	3 mL × 1 vial	-20°C, 12 months, shading light
Reagent 9	8 mmol/L Standard Solution	0.2 mL × 1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

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

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **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°C for one day protected from light or -20°C for 1 month.
3. **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°C for one day protected from light or -20°C for 1 month.

- 4. 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°C for 7 days protected from light.
- 5. 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 EP tube, mix well. The measuring working solution should be prepared on spot and used up within 30 min.
- 6. 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°C for 7 days protected from light.
- 7. 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.

7. Sample preparation

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°C.
4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10⁶ cells).
2. Wash cells in 200 µL normal saline (0.9% NaCl).
3. Homogenize 1×10⁶ cells in 200 µL extraction solution with an ultrasonic cell disruptor at 4°C.
4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

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

1×10⁶ CHO cells: 1

1×10⁶ 293T cells: 1

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

8. 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.

9. 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°C 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 F1. Stand at 25°C for 30 min. Measure the fluorescence intensity at the

excitation wavelength of 535 nm and the emission wavelength of 587 nm, as F2.
(The standard curve is fitted to the standard well in F1.)

10. 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 absolute fluorescence value.
3. Plot the standard curve by using absolute 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 catalyzes the substrate to produce 1 μ mol product at 25°C for 1 min is defined as 1 unit.

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

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

2. Animal tissue and cell samples:

Definition: the amount of enzyme in 1 g of tissue or cell protein that catalyzes the substrate to produce 1 μ mol product at 25°C for 1 min is defined as 1 unit.

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

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

[Note]

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

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

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.

11. Appendix

Performance Characteristics

Intra-assay Precision

Three mouse serum 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)	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.

Inter-assay Precision

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%.

Recovery

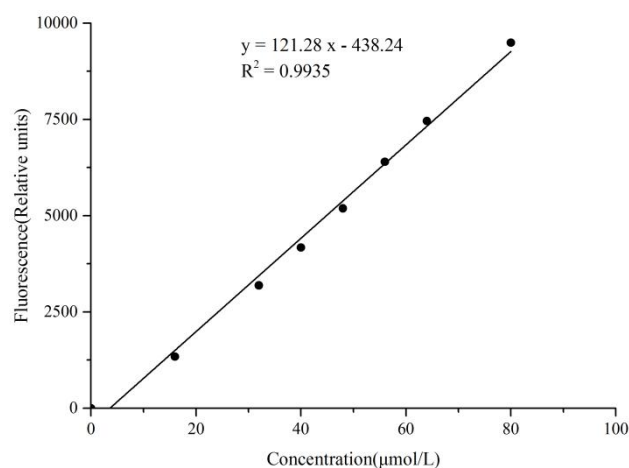
Sample	Expected Conc. (U/L)	Observed Conc. (U/L)	Recovery rate (%)
Sample 1	1.0	0.96	96
Sample 2	1.5	1.6	106
Sample 3	2	2.2	110

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.

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:



Standard curve

Concentration (μmol/L)	Fluorescence value 1	Fluorescence value 2	Average fluorescence value	Absolute fluorescence value
0	238	200	219	0
16	1517	1603	1560	1341
32	3407	3407	3407	3189
40	4505	4277	4391	4172
48	5492	5327	5410	5191
56	6598	6638	6618	6399
64	7727	7624	7675	7457
80	9626	9802	9714	9495

Example Analysis

Example analysis:

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

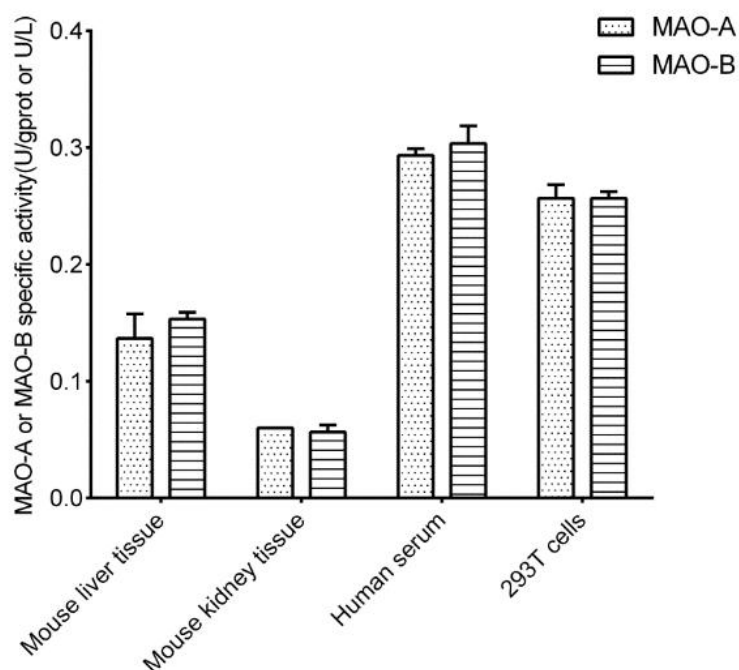
Standard curve: $y = 121.28x - 438.24$, the average F_1 of the control well is 197, the average F_2 of the control well is 488, the average F_1 of the MAO-A sample well is 192, the average F_2 of the MAO-A sample well is 394, the average F_1 of the MAO-B sample well is 200, the average F_2 of the MAO-B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

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

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

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

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:



12. 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 the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. 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.