TECHNICAL MANUAL

Monoamine Oxidase (MAO) Activity Assay Kit

- **SKU CODE:** MAES0021
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Sample preparation
- Reagent preparation
- Add samples and reagents
- Measure initial absorbance (A1)
- Incubate at 37°C for 30 min
- Measure final absorbance (A2)
- Calculate MAO activity

2. Intended use

This kit can be used to measure monoamine oxidase (MAO) activity in animal tissue samples.

3. Detection principle

MAO catalyzes 4-dimethylaminobenzylamine to produce p-dimethylaminobenzaldehyde. p-Dimethylaminobenzaldehyde has a characteristic absorption peak at 355 nm. The activity of MAO can be calculated indirectly by measuring the production of p-dimethylaminobenzaldehyde.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution A	30 mL × 1 vial	60 mL × 1 vial	2-8°C, 12 months
Reagent 2	Extraction Solution B	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 3	Buffer Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 4	Chromogenic Agent	3 mL × 1 vial	5 mL × 1 vial	2-8°C, 12 months
	UV Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Centrifuge, Microplate reader (345-360 nm, optimum wavelength: 355 nm), Micropipette, Incubator

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of extraction solution A working solution:** Dilute 100 µL of Extraction Solution A with 100 µL of double distilled water, mix well. Store at 2-8°C for 1 month.
3. **Preparation of buffer working solution:** For each well, prepare 150 µL of buffer working solution by mixing 75 µL of Buffer Solution with 75 µL of double distilled water. Mix well. Store at 2-8°C for 1 month.

7. Sample preparation

1. **Tissue sample preparation:** 1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg). 2. Wash tissue in cold PBS (0.01 M, pH 7.4). 3. Homogenize 20 mg tissue in 180 µL extraction solution A working solution with a dounce homogenizer at 4°C. 4. Centrifuge at 1000×g at 4°C for 10 min to remove insoluble material. (Note: determine the protein concentration of supernatant before centrifugation). 5. Collect supernatant and centrifuge at 10000×g at 4°C for 30 min, discard the supernatant and keep the pellet. 6. Add 1 mL of pre-cooled Extraction Solution B and mix thoroughly. Centrifuge at 16000×g at 4°C for 40 min, discard the supernatant and keep the pellet. 7. Add 1 mL of pre-cooled buffer working solution and mix thoroughly. Keep on ice during use.
2. **Sample dilution:** The recommended dilution factor for different samples (for reference only): • 10% Mouse liver tissue homogenate: 1 • 10% Rat liver tissue homogenate: 1 • 10% Rat lung tissue homogenate: 1 • 10% Rat kidney tissue homogenate: 1 • 10% Mouse brain tissue homogenate: 1 Note: The diluent is buffer working solution. For dilution of other sample types, perform a pre-test to confirm the dilution factor.

8. Key points of the assay

1. Prevent bubble formation when transferring supernatant into the microplate.

2. Use UV microplate for detection.

3. During tissue sample pretreatment, pre-cool extraction solution A working solution, extraction solution B, and buffer working solution for 30 min in advance.

4. During operating steps, pre-heat buffer working solution and chromogenic agent at 37°C for 30 min in advance.

5. For tissue samples, determine the protein concentration separately.

9. Operating steps

1. Add 25 µL of sample to corresponding sample wells.

2. Add 150 µL of buffer working solution to sample wells.

3. Add 25 µL of chromogenic agent to sample wells and mix thoroughly with microplate reader for 5 s.

4. Measure the OD values of each well at 355 nm with microplate reader, record as A1, then incubate at 37°C for exactly 30 min.

5. Measure the OD values of each well again, record as A2.

10. Calculation

Tissue sample:

Definition: The amount of enzyme in 1 g of tissue protein that catalyzes the substrate to produce 1 nmol p-dimethylaminobenzaldehyde at 37°C for 1 min is defined as 1 unit.

MAO activity (U/g_{prot}) = $(A_2 - A_1) / (\varepsilon \times d) \times V_1 \div (V_2 \times C_{pr}) \div T \times f$

Where:

A_1: The initial absorbance of the sample

A_2: The absorbance of the sample after 30 min incubation

T: The incubation time, 30 min

ε: The molar extinction coefficient of p-dimethylaminobenzaldehyde, 2.77×10^{-4} L/(nmol·cm)

d: The optical path of microplate, 0.6 cm

V_1: The total reaction volume, 200 µL

V_2: The sample volume added to the reaction, 25 µL

C_{pr}: The protein concentration in sample, g_{prot}/L

f: Sample dilution factor before testing

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three rat liver tissue samples 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)	25.00	240.00	350.00
%CV	3.5	3.1	3.3

Inter-assay Precision

Three rat liver tissue samples 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)	25.00	240.00	350.00
%CV	5.7	6.2	6.1

Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 100%.

Recovery

Sample	Expected Conc. (U/L)	Observed Conc. (U/L)	Recovery rate (%)
Sample 1	90	92.7	103
Sample 2	285	305.0	107

Sample	Expected Conc. (U/L)	Observed Conc. (U/L)	Recovery rate (%)
Sample 3	460	483.0	105

Sensitivity

The analytical sensitivity of the assay is 16 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.

12. Appendix II Example Analysis

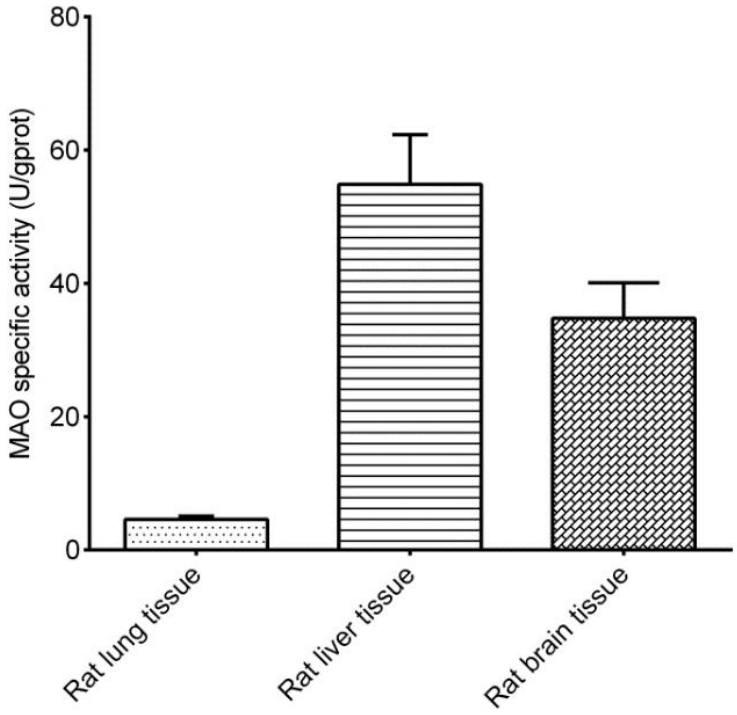
Example analysis:

For rat liver tissue, 25 μL of 10% rat liver tissue homogenate was assayed according to the operating steps. The results are as follows:

The initial OD value of the sample (A_1) is 0.684, the OD value of the sample after 30 min (A_2) is 1.016, the protein concentration in sample is 11.27 g_{prot}/L, and the calculation result is:

MAO activity (U/g_{prot}) = (1.016-0.684) ÷ (2.77×10^{-4}) ÷ 0.6 × 200 ÷ (11.27 × 25) ÷ 30 = 47.26 U/g_{prot}

Detection of 10% rat lung tissue homogenate (protein concentration: 6.14 g_{prot}/L), 10% rat liver tissue homogenate (protein concentration: 11.27 g_{prot}/L), 10% rat brain tissue homogenate (protein concentration: 4.33 g_{prot}/L) according to the protocol showed the following results:



13. 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!

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.