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

Catalog No：E-BC-K008-M

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

Measuring instrument：Microplate reader（345-360 nm）

Detection range：16-641 U/L

Elabscience® Monoamine Oxidase (MAO) Activity Assay Kit

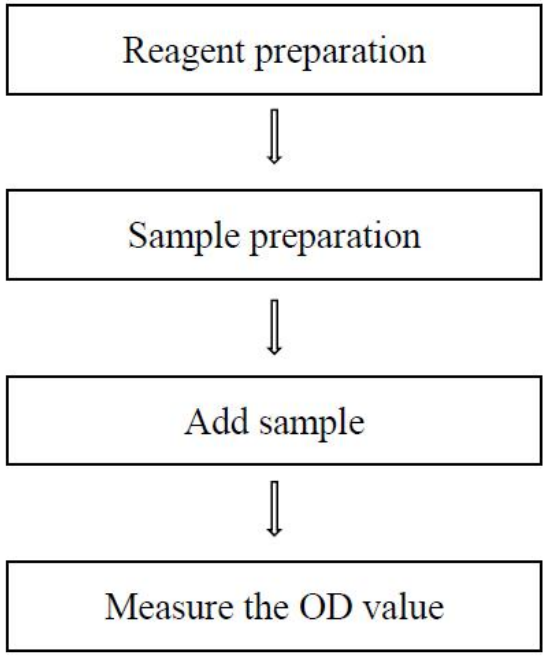
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 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 animal tissue samples.

Detection principle

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

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		

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of extraction 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 The preparation of buffer working solution:

For each well, prepare 150 μL of buffer working solution (mix well 75 μL of buffer solution and 75 μL of double distilled water). Store at 2-8°C for 1 month.

Sample preparation

① Sample preparation

Tissue sample:

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 (E-BC-K318-M) before centrifugation),

⑤ Collect supernatant and centrifuge at 10000×g at 4°C.for 30 min, discard the supernatant and keep the sediment.

⑥ Add 1 mL of pre-cooled extraction solution B and mix fully. Centrifuge at 16000×g at 4°C for 40 min, discard the supernatant and keep the sediment.

⑦ Add 1 mL of pre-cooled buffer working solution and mix fully, keep it on ice during use.

② Dilution of sample

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

Sample type	Dilution factor
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 solution working 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 the supernatant is transferred into the microplate.

② UV microplate was used for detection.

③ During the tissue sample pretreatment step, extraction A working solution, extraction solution B and buffer working solution need to be pre-cooled for 30 min in advance

④ During the operation steps, buffer working solution and chromogenic agent need to be pre-heated at 37°C for 30 min in advance.

⑤ For tissue sample, the protein concentration in sample should be determine separately (E-BC-K318-M).

Operating steps

① Sample well: 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 fully with microplate reader for 5 s.

④ Measure the OD values of each well at 355 nm with microplate reader, recorded as A_1, and then incubate accurately at 37°C for 30 min, measure the OD values of each well again, recorded as A_2.

Calculation

The sample:

Tissue sample:

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

$$\text{MPO activity (U/gprot)} = \frac{(A_2 - A_1)}{\varepsilon \times d} \times V_1 \div (V_2 \times C_{pr}) \div T \times f$$

[Note]

A_1: The initial absorbance of the sample.

A_2: The absorbance of the sample after incubate for 30 min.

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

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

d: The optical path of cuvette, 0.6 cm.

V_1: The total volume of reaction, 200 μL.

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

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

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. 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)

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.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	25.00	240.00	350.00
%CV	5.7	6.2	6.1

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%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	90	285	460
Observed Conc. (U/L)	92.7	305.0	483.0
Recovery rate (%)	103	107	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.

Appendix II Example Analysis

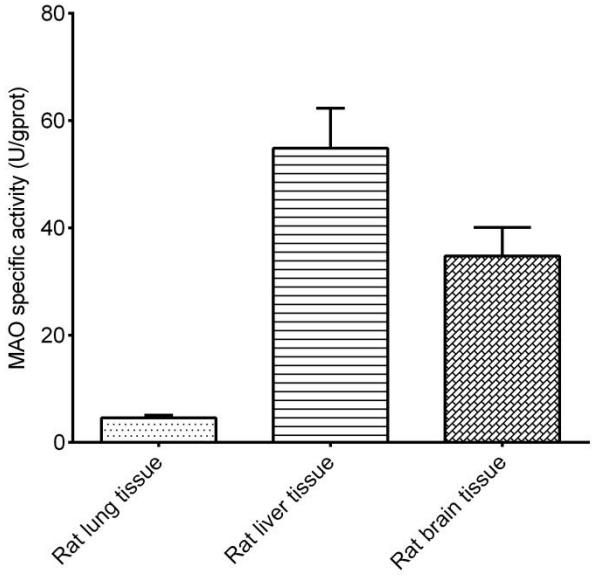
Example analysis：

For rat liver tissue, take 25 μL of 10% rat liver tissue homogenate, and carry the assay according to the operation 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 concentration of protein in sample is 11.27 gprot/L, and the calculation result is:

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

Detect 10% rat lung tissue homogenate (the concentration of protein is 6.14 gprot/L), 10% rat liver tissue homogenate (the concentration of protein is 11.27 gprot/L), 10% rat brain tissue homogenate (the concentration of protein is 4.33 gprot/L) and porcine serum 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.