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

Catalog No：E-BC-K008-S

Specification：50Assays (49 samples)/100Assays (99 samples)

Measuring instrument：Spectrophotometer（355 nm）

Detection range：6-722 U/L

Elabscience®Monoamine Oxidase (MAO) Activity Assay Kit

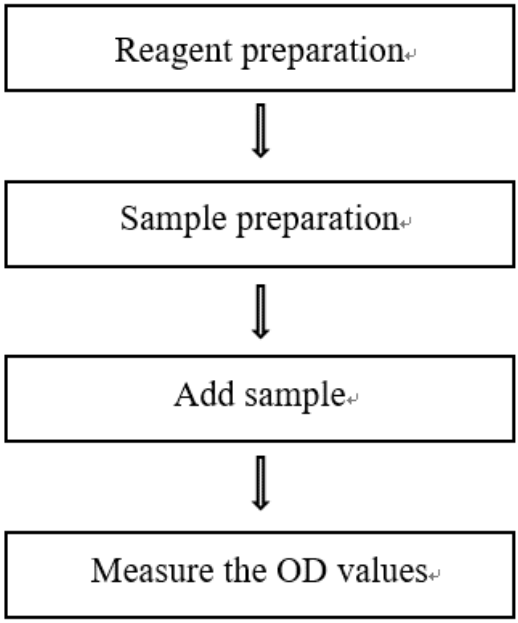
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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

Monoamine oxidase (MAO, EC1.4.3.4) can catalysis 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 (50Assays)	Size 2 (100Assays)	Storage
Reagent 1	Extraction Solution A	30 mL ×1 vial	60 mL ×1 vial	2-8℃, 12 months
Reagent 2	Extraction Solution B	60 mL ×1 vial	60 mL ×2 vials	2-8℃, 12 months
Reagent 3	Buffer Solution	60 mL ×1 vial	60 mL ×2 vials	2-8℃, 12 months
Reagent 4	Chromogenic Agent	10 mL ×1 vial	15 mL ×1 vial	2-8℃, 12 months

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:

Microplate reader (355 nm), Micropipettor, Vortex mixer, Incubator, Tubes

Reagents:

Double distilled water

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of extraction working solution A:

For each tube, prepare 100 μL of extraction working solution A (mix well 50 μL of extraction solution A and 50 μL of double distilled water). Store at 2-8℃ for 1 month.

③ The preparation of buffer working solution:

For each tube, prepare 1000 μL of buffer working solution (mix well 500 μL of extraction solution A and 500 μL of double distilled water). Store at 2-8℃ 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℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and determine the protein concentration of supernatant (E-BC-K318-M).

⑤ Take the supernatant, and centrifuge at 10000×g for 30 minutes to remove supernatant. Collect sediment and add 0.2 mL of pre-cooled extraction solution B, mix well.

⑥ Centrifuge at 16000×g for 40 minutes to remove supernatant. Collect sediment and add 0.2 mL of pre-cooled buffer solution, mix well. Keep it on ice for detection.

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% Mouse kidney tissue homogenate	1
10% Rat brain tissue homogenate	1
Human serum	1

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

The key points of the assay

① During the tissue sample pretreatment step, extraction working solution A, extraction solution B and buffer working solution need to be pre-cooled for 30 minutes in advance.

② During the operation steps, buffer working solution and chromogenic agent need to be pre-heated at 37℃ for 30 min in advance.

③ If the monoamine oxidase content is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M).

Operating steps

① Sample tube: add 100 μL of sample to the tube.
Blank tube: add nothing.

② Sample tube: add 800 μL of buffer working solution to tube.
Blank tube: add 1000 μL of buffer working solution to tube.

③ Sample tube: add 100 μL of chromogenic agent to the tube.
Blank tube: add nothing.

④ Set the spectrophotometer to zero with blank tube and measure the OD value of sample tube with 1 cm optical path cuvette at 355 nm, recorded as A_1, and then incubate accurately at 37℃ for 30 min, measure the OD values of each tube 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 ℃ 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]

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, 1 cm.

V_1: The total volume of reaction, 1 mL.

V_2: The volume of sample 0.1 mL.

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 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)	80.00	265.00	420.00
%CV	3.2	2.4	2.5

Inter-assay Precision

Three human serum 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)	80.00	265.00	420.00
%CV	3.5	3.0	3.4

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 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/L)	130	325	510
Observed Conc.(U/L)	130.0	331.5	515.1
Recovery rate (%)	100	102	101

Sensitivity

The analytical sensitivity of the assay is 6 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:

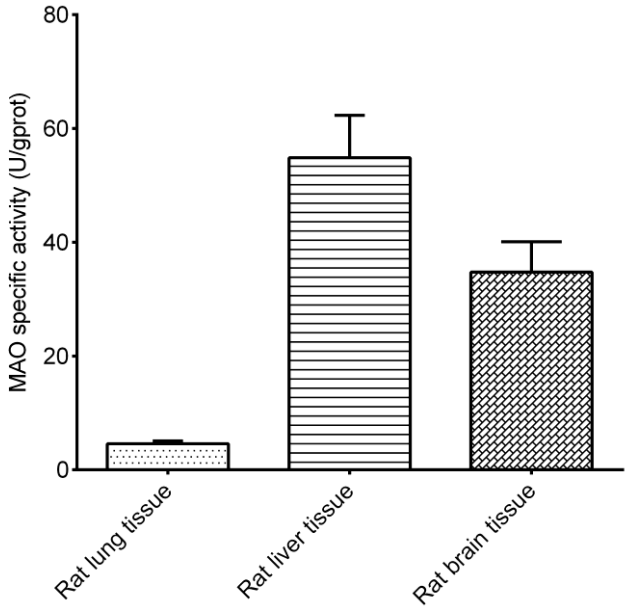
For rat liver tissue, take 100 μL of 10% rat liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

the initial OD value of the sample (A_1) is 0.743, the OD value of the sample after incubate for 30 min (A_2) is 1.335, the concentration of protein in sample is 11.27 gprot/L, and the calculation result is:

$$\text{MAO activity (U/gprot)} = (1.335\text{-}0.743) \div (2.77 \times 10\text{-}4) \div 1 \times 1 \div (0.1 \times 11.27) \div 30$$

$$= 63.21 \text{ U/gprot}$$

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 human 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.