Monoamine Oxidase (MAO) Activity Assay Kit

Catalog No: E-BC-K008-S

Method: Colorimetric method

Specification: 100Assays (Can detect 99 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 6 U/L

Detection range: 6-722 U/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

Monoamine oxidase (MAO, EC1.4.3.4) is a "flavin protein" containing α-mercaptopropionicacid in the outer membrane of mitochondria of cells. Its main function in vivo is to catalyze the metabolism of endogenous and exogenous monoamine substances. Under the action of MAO, monoamines are oxidized to produce deamination. MAO is ubiquitous in central nervous system and nerve endings, and it is mainly located on the outer membrane of mitochondria of tissues such as brain, liver and intestinal mucosa.

▲Detection principle

MAO can catalysis 4-dimethylambenzylamine to produce p-dimethylaminoben -zaldehyde. 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	Specification	Storage
Reagent 1	Extraction Solution A	60 mL × 1 vial	2-8℃ , 6 months
Reagent 2	Extraction Solution B	60 mL × 2 vials	2-8℃ , 6 months
Reagent 3	Buffer Solution	60 mL × 2 vials	2-8℃ , 6 months
Reagent 4	Chromogenic Agent	15 mL × 1 vial	2-8℃ , 6 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.			

▲Materials prepared by users

Instruments

Spectrophotometer (355 nm), Micropipettor, Vortex mixer, Incubator, Tubes

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. During the tissue sample pretreatment step, reagent 1 working solution, reagent 2 and reagent 3 working solution need to be pre-cooled for 30 minutes in advance.
2. During the operation steps, reagent 3 working solution and reagent 4 need to be pre-heated at 37°C for 30 min in advance.
3. For tissue sample, the protein concentration in sample should be determine separately (E-BC-K318-M).

Pre-assay preparation

▲Reagent preparation

1. The preparation of reagent 1 working solution:
   Mix reagent 1 with double distilled water fully at a ratio of 1:1. The prepared solution can be stored at 2-8℃ for 1 month.
2. The preparation of reagent 3 working solution:
   Mix reagent 3 with double distilled water fully at a ratio of 1:1. The prepared solution can be stored at 2-8℃ for 1 month.

▲Sample preparation

1. Serum (plasma): detect directly.
2. 10% tissue homogenate: Accurately weigh the tissue sample, add 9 times the volume of pre-cooled reagent 1 working solution according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 1000 g for 10 min (Note: determine the protein concentration of supernatant (E-BC-K318-M) before centrifugation), then take the supernatant and centrifuge at 10000 g at 4°C for 30 min, discard the supernatant and keep the sediment. Add 1 mL of pre-cooled reagent 2 and mix fully, centrifuge at 16,000 g at 4°C for 40 min, discard the supernatant and keep the sediment. Finally, add 1 mL of pre-cooled reagent 3 working solution, mix fully, and preserve it on ice for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (6-722 U/L).
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 reagent 3 working solution.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	355 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. Sample tube: Add 100 μL of sample to 1.5 mL tube.
   Blank tube: Add nothing.
2. Sample tube: Add 800 μL of reagent 3 working solution to the tube.
   Blank tube: Add 1000 μL of reagent 3 working solution to the tube.
3. Sample tube: Add 100 of reagent 4 to sample 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°C for 30 min, measure the OD values of each tube again, recorded as A_2.

▲Operation table

	Sample tube	Blank tube
Sample (μL)	100	
Reagent 3 working solution (μL)	800	1000
Reagent 4 (μL)	100	
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°C for 30 min, measure the OD values of each tube again, recorded as A_2.		

▲Calculation

1. Serum (plasma) sample:

Definition: the amount of enzyme in 1 L of serum (plasma) that catalyze the substrate to produce 1 nmol p-dimethylaminobenzaldehyde at 37 ℃ for 1 min is defined as 1 unit.

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

2. 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.

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

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	6-722 U/L	Average intra-assay CV (%)	2.7
Sensitivity	6 U/L	Average inter-assay CV (%)	3.3
Average recovery rate (%)	101		

▲Example analysis

For rat liver tissue, take 100 μL of 10% rat liver tissue homogenate, and carry the assay according to the operation table.

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:

$$\text{MAO activity U/gprot} = \frac{(1.335-0.743)}{(2.77\times10^{-4})} \div 1 \times 1 \div (0.1\times11.27) \div 30 = 63.21 \text{U/gprot}$$