Monoamine Oxidase (MAO) Activity Assay Kit

Catalog No: E-BC-K008-M

Method: Colorimetric method

Specification: 96T (Can detect 96 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 16 U/L

Detection range: 16 – 641 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 the 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 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	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	5 mL × 1 vial	2-8℃ , 6 months
	Microplate	96 wells	
	Plate Sealer	2 pieces	

▲Materials prepared by users

Instruments

Centrifuge, Microplate reader (345-360 nm), Micropipette, Incubator.

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. 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, reagent 1 working solution, reagent 2 and reagent 3 working solution need to be pre-cooled for 30 minutes in advance.
4. During the operation steps, reagent 3 working solution and reagent 4 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).

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 (16 – 641 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% Rat kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
Human serum	1

[Note]：The diluent is reagent 3 working solution.

Assay protocol	
Ambient temperature	25-30℃
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

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94
G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87	S95
H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88	S96

[Note]：S1-S96, sample wells.

▲Operating steps

1. The measurement of samples

1) Sample well: Add 25 μL of sample to corresponding sample wells.
2) Add 150 μL of reagent 3 working solution to sample wells.
3) Add 25 μL of reagent 4 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℃ for 30 min, measure the OD values of each well again, recorded as A_2.

▲Operation table

	Sample well
Sample (μL)	25
Reagent 3 working solution (μL)	150
Reagent 4 (μL)	25
Mix fully with microplate reader for 5 s. Measure the OD values of each well at 355 nm with microplate reader, recorded as A_1, and then incubate accurately at 37℃ for 30 min, measure the OD values of each well again, recorded as A_2.	

▲Calculation

1. Serum (plasma) and other liquid 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.

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

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℃ for 1 min is defined as 1 unit.

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

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, 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.

▲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	16 – 641 U/L	Average inter-assay CV (%)	3.3
Sensitivity	16 U/L	Average inter-assay CV (%)	6.0
Average recovery rate (%)	105		

▲Example analysis

For rat liver tissue, take 25 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 0.684, 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