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

Catalog No：E-BC-D012

Specification：48T/96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Elabscience® Monoamine Oxidase A (MAO-A) Inhibitor Screening 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

Intended use

This kit is used for the determination of the inhibitory effect of monoamine oxidase A (MAO-A) inhibitors.

Detection principle

Monoamine Oxidase (MAO) is a mitochondrial membrane binding enzyme, which is widely present in connective tissues and cell mitochondria of liver, kidney, small intestine, stomach, brain and other organs. MAO-A metabolizes neurotransmitters such as serotonin, dopamine, serotonin, epinephrine and norepinephrine. MOA inhibition increases levels of neurotransmitters and are used to treat depression, neurodegenerative diseases, and cardiovascular disease.

The detection principle of this kit: MAO-A can catalyze the substrate to produce fluorescent substances. After adding inhibitors, the generation of fluorescent substances will be inhibited, and the effect of inhibitors will be determined according to the degree of inhibition.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months, shading light
Reagent 2	Enzyme Diluent	1.2 mL × 1 vial	1.2 mL × 2 vials	-20°C, 12 months
Reagent 3	Enzyme Reagent A	0.03 mL × 1 vial	0.06 mL × 1 vial	-20°C, 12 months
Reagent 4	Substrate	0.21 mL × 1 vial	0.42 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	Enzyme Reagent B	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
Reagent 6	Probe	0.03 mL × 1 vial	0.06 mL × 1 vial	-20°C, 12 months, shading light
Reagent 7	0.2 mmol/L Clorgyline Hydrochloride	0.2 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months, shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Incubator (37℃),

Reagents:

DMSO

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of enzyme A diluent solution：
Before testing, please prepare sufficient enzyme A diluent solution. For example, prepare 200 μL of enzyme A diluent solution (mix well 195 μL of enzyme diluent and 5 μL of enzyme reagent A). Store at -20°C for 3 days.

③ The preparation of enzyme A working solution:
For each well, prepare 60 μL of enzyme A working solution (mix well 40 μL of buffer solution and 20 μL of enzyme A diluent solution). The enzyme A working solution should be prepared on spot and used up within the same day.

④ The preparation of enzyme B working solution:
Dissolve one vial of enzyme reagent B with 275 μL of double distilled water, mix well to dissolve. Store at 2-8°C for 7 days protected from light.

⑤ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 1745 μL of working solution (mix well 1600 μL of buffer solution, 40 μL of substrate, 100 μL of enzyme B working solution and 5 μL of probe). The working solution should be prepared on spot and used up within 30 min.

⑥ The preparation of clorgyline hydrochloride working solution:
The concentrate of clorgyline hydrochloride provided with this kit is 0.2 mmol/L and can be diluted to the desired concentration with DMSO (This reagent is a MAO-A inhibitor, as a positive control, the determination of inhibition rate can be used as a reference).

Sample preparation

It is recommended to dilute the sample with buffer solution. If the sample is poorly water-soluble, DMSO can be prepared into a high-concentration solution and then diluted with buffer solution. The DMSO content in the reaction system should be less than 5%.

The key points of the assay

① The volume of enzyme reagent A and probe is samll and need to be centrifuged before use.

② The reaction will start immediately after adding the substrate. It is recommended to use the multichannel pipeter when the number of samples is large.

Operating steps

① Blank well: Add 80 μL of buffer solution to the corresponding wells. Total enzyme well: Add 20 μL of buffer solution to the corresponding wells.

② Add 60 μL of enzyme A working solution to total enzyme wells, positive control wells and sample wells.

③ Add 20 μL of clorgyline hydrochloride working solution into positive control wells.

④ Add 20 μL of sample into sample wells.

⑤ Incubate at room temperature for 30 min.

⑥ Add 160 μL of working solution into each well.

⑦ Mix fully with fluorescence microplate reader for 5 s. Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, as F_1. Incubate at 37ºC for 10 min and measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm, as F_2. $\Delta F=F_2-F_1$

Calculation

$$\text{Inhibition Rate (\%)} = (\Delta F_{total} - \Delta F_{sample}) \div (\Delta F_{total} - \Delta F_{blank}) \times 100\%$$

[Note]

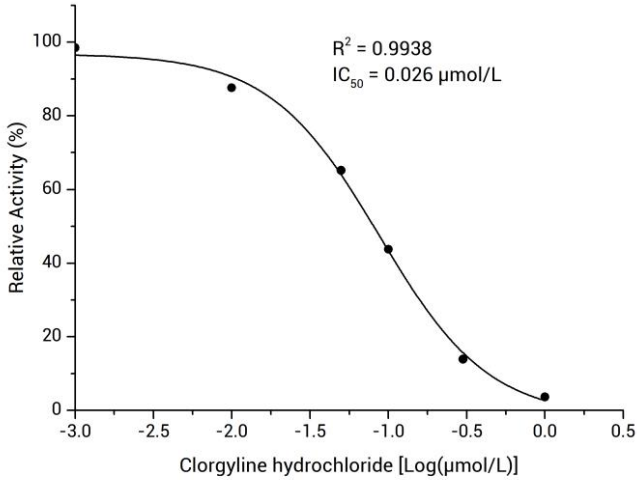
ΔF_{total}: The absoluted fluorescence intensity of total enzyme well, $\Delta F = F_2 - F_1$.

ΔF_{sample}: The absoluted fluorescence intensity of sample well, $\Delta F = F_2 - F_1$.

ΔF_{blank}: The absoluted fluorescence intensity of blank well, $\Delta F = F_2 - F_1$.

Appendix I Performance Characteristics

Effect diagram of monoamine oxidase A(MAO-A) inhibitor screening kit for detecting MAO-A inhibitor chlorjiline hydrochloride.



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.