Monoamine Oxidase Inhibitor Screening Kit (Fluorometric)

Monoamine Oxidase Inhibitor Screening Kit (Fluorometric) is a screening kit for inhibitor screening and evaluation of monoamine oxidase inhibitors.

Catalog number: ARG82184

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INTRODUCTION

Monoamine oxidases (MAO) (EC 1.4.3.4) are a family of enzymes that catalyze the oxidation of monoamines, employing oxygen to clip off their amine group. They are found bound to the outer membrane of mitochondria in most cell types of the body. The first such enzyme was discovered in 1928 by Mary Bernheim in the liver and was named tyramine oxidase. The MAOs belong to the protein family of flavin-containing amine oxidoreductases.

MAOs are important in the breakdown of monoamines ingested in food, and also serve to inactivate monoamine neurotransmitters. Because of the latter, they are involved in a number of psychiatric and neurological diseases, some of which can be treated with monoamine oxidase inhibitors (MAOIs) which block the action of MAOs.

Because of the vital role that MAOs play in the inactivation of neurotransmitters, MAO dysfunction (too much or too little MAO activity) is thought to be responsible for a number of psychiatric and neurological disorders. For example, unusually high or low levels of MAOs in the body have been associated with schizophrenia, depression, attention deficit disorder, substance abuse, migraines, and irregular sexual maturation. Monoamine oxidase inhibitors are one of the major classes of drug prescribed for the treatment of depression, although they are often last-line treatment due to risk of the drug's interaction with diet or other drugs. Excessive levels of

catecholamines (epinephrine, norepinephrine, and dopamine) may lead to a hypertensive crisis, and excessive levels of serotonin may lead to serotonin syndrome.

In fact, MAO-A inhibitors act as antidepressant and antianxiety agents, whereas MAO-B inhibitors are used alone or in combination to treat Alzheimer's disease and Parkinson's disease. Some research suggests that certain phenotypes of depression, such as those with anxiety, and "atypical" symptoms involving psychomotor retardation, weight gain and interpersonal sensitivity. However the findings related to this have not been consistent. MAOIs may be effective in treatment resistant depression, especially those that do not respond to tricyclic antidepressants. [Wikipedia]

PRINCIPLE OF THE ASSAY

Monoamine Oxidase Inhibitor Screening Kit provides a convenient fluorometric material to screen MAO enzyme inhibitors. In this assay, Monoamine Oxidase reacts with *p*-tyramine, a substrate for both MAO-A and MAO-B, resulting in H_2O_2 formation. And then H_2O_2 is determined by a fluorometric method (λem/ex = 585/530 nm). The assay is simple, sensitive, stable and high throughput adaptable.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	12 ml (Ready to use)	-20°C
MAO-A inhibitor Clorgyline (20mM)	50 µl	-20°C
MAO-B inhibitor Pargyline (20mM)	50 µl	-20°C
p-Tyramine	120 µl	-20°C
HRP Enzyme	120 µl	-20°C
Dye Reagent	120 µl	-20°C

The kit is shipped on ice. Store all components at -20°C in dark. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence Microplate Reader capable of measuring fluorescence at λ em/ex = 585/530 nm.
- Black flat bottomed 96-well microplate
- Pipettes and pipette tips
- Dounce homogenizer (for tissue samples)
- Deionized or distilled water.
- Enzyme MAO-A or MAO-B (MAO-A: 3 U/mL and MAO-B: 6 U/mL)

TECHNICAL HINTS AND PRECAUTIONS

- This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick and mixing should be brief but thorough.
- Use of a multichannel pipettor is recommended.
- Neither the enzyme MAO-A nor MAO-B is included in the kit.
- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Prior to assay, concentrations of protein, inhibitor, substrate and incubation time may need to be established for a given sample.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Notice:

1. thiols (β-mercaptoethanol, dithioerythritol etc) at > 10 µM interfere with this assay and should be avoided in sample preparation.
2. It is recommended aliquot and store enzyme sample at-80°C.

3. Dilute purified MAO-A to 3 U/mL and MAO-B to 6 U/mL using distilled water.
4. Dissolve the test compounds in solvent of choice. It is prudent to first test the tolerance of the solvent by the enzyme of choice.
5. If DMSO is used, its concentration in the 5 µl of test compounds added to the reaction should be less than 10% (v/v) when screening with human MAO inhibitor.

REAGENT PREPARATION

Notice: the following protocol and reagent preparation instructions are optimized for human MAO. If another species is being analyzed, we recommend that user experimentally determine the Km and then adjust the volume of substrate in the Working reagent so that the final concentration of the substrate in the 50 µl reaction is near the Km.

- p-Tyramine: For human MAO-A, use a 1.5X dilution of the provided p-Tyramine by adding 80 µl p-Tyramine to 40 µl dH2O. For human MAO-B, use a 4X dilution of the provided p-Tyramine by adding 30 µl p-Tyramine to 90 µl dH2O.
- MAO-A inhibitor Clorgyline / MAO-B inhibitor Pargyline: Dilute the 20 mM inhibitors with distilled water to 10 µM. (e.g. mix 5 µl of 20 mM inhibitor with 9.995 ml of distilled water).
- Assay Working Reagent: *Prepare before use,* mix 50 µl of Assay Buffer, 1 µl of diluted p-tyramine (for MAO-A, dilute 1.5X; for MAO-B, dilute 4X) 1 µl of Dye Reagent and 1 µl of HRP Enzyme. Transfer 50 µl of Working

Reagent to each wells. Using the prepared Working Reagent within 15 min.

- Blank Working Reagent: *Prepare before use,* mix 50 µl of Assay Buffer, 1 µl of distilled water, 1 µl of Dye Reagent and 1 µl of HRP Enzyme. Transfer 50 µl of Working Reagent to Blank wells.
- Assay buffer: Assay buffer is ready to use, mix it well by vigorous shaking before use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use. Use black flat-bottom plates for the assay.

For MAO-A inhibitor screening:

1. Add 45 µl of purified MAO-A (3 U/mL) in two wells and add 5 µl of solvent that the test compounds are dissolved in, one for blank and the other one for No Inhibitor control well. (For example, if the test compounds are dissolved in 10% (v/v) DMSO, add 5 µl 10% (v/v) DMSO to these wells.
2. Add 45 µl of purified MAO-A (3 U/mL) in one well and add 5 µl of 10 µM clorgyline as MAO-A positive inhibitor control wells.
3. Add 45 µl of purified MAO-A (3 U/mL) in other wells and add 5 µl of test compounds as sample wells.
4. Gently tap the plate to ensure thorough mixing. Incubate for 15 min at room temperature (25°C) for the inhibitor to block MAO-A activity.
5. Prepare enough MAO-A Assay Working Reagent for all wells except the

Blank well.

6. Add 50 µl of MAO-A Assay Working Reagent (with 1.5X diluted p-tyramine) into all wells except the Blank well.
7. Add 50 µl of Blank Working Reagent (without p-tyramine) into Blank well.
8. Incubation the plate in dark at the room temperature (25°C) for 20 min.
9. Read fluorescence intensity at λ ex = 530 nm and λ em = 585 nm.

For MAO-B inhibitor screening:

1. Add 45 µl of purified MAO-B (6 U/mL) in two wells and add 5 µl of solvent that the test compounds are dissolved in, one for **blank** and the other one for **No Inhibitor control** well. (For example, if the test compounds are dissolved in 10% (v/v) DMSO, add 5 µl 10% (v/v) DMSO to these wells.
2. Add 45 µl of purified MAO-B (6 U/mL) in one well and add 5 µl of 10 µM pargyline as **MAO-B positive inhibitor control** wells.
3. Add 45 µl of purified MAO-B (6 U/mL) in other wells and add 5 µl of test compounds as **test sample** wells.
4. Gently tap the plate to ensure thorough mixing. Incubate for 15 min at room temperature (25°C) for the inhibitor to block MAO-B activity.
5. Prepare enough MAO-B Assay Working Reagent for all wells except the Blank well.
6. Add 50 µl of MAO-A Assay Working Reagent (with 4X diluted p-tyramine) into all wells except the Blank well.
7. Add 50 µl of Blank Working Reagent (without p-tyramine) into Blank well.
8. Incubation the plate in dark at the room temperature (25°C) for 20 min.

9. Read fluorescence intensity at λ ex = 530 nm and λ em = 585 nm.

Protocol Summary

	Blank	No Inhibitor control	Positive control	Test samples
MAO-A (3 U/mL) / MAO-B (6 U/mL)	45 µl	45 µl	45 µl	45 µl
solvent	5 µl	5 µl		
10 µM clorgyline / 10 µM pargyline			5 µl	
Test compounds				5 µl
Incubation the plate at the RT (25°C) for 15 min				
Assay Working Reagent		50 µl	50 µl	50 µl
Blank Working Reagent	50 µl			
Incubation the plate in dark at the RT (25°C) for 20 min				
Read fluorescence intensity at λ ex = 530 nm and λ em = 585 nm				

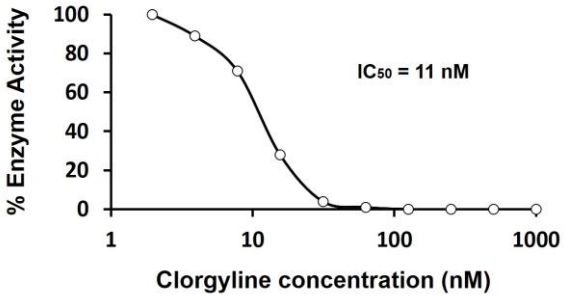
CALCULATION OF RESULTS

The percent of MAO activity in the presence of a test compound is calculated as follows:

% Activity =

$[(RFU_{test\ sample} - RFU_{Blank}) / (RFU_{No\ Inhibitor} - RFU_{Blank})] \times 100\%$

EXAMPLE OF INHIBITOR TITRATIONS:



Human MAO-A and MAO-B were incubated with various concentrations of clorgyline. The IC50 for Clorgyline with 3 U/mL human MAO-A was determined to be 11 nM; while the IC50 for pargyline with 6 U/mL human MAO-B was determined to be 404 nM.