Monoamine Oxidase Activity Assay Kit (Fluorometric)

Monoamine Oxidase Activity Assay Kit (Fluorometric) can be used to measure Monoamine Oxidase activity in biological samples and it also can be used to evaluate and screen for MAO inhibitors.

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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

This Monoamine Oxidase Activity Assay Kit provides a convenient fluorometric material to measure Monoamine Oxidase enzyme activity. 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
3% H_2O_2 standard	100 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 (for human MAO-A and MAO-B: MAO-A: 3 U/mL and MAO-B: 6 U/mL)

TECHNICAL HINTS AND PRECAUTIONS

- 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. Biological fluids may be assayed directly. Samples should be free of particle or precipitates. Remove particle or precipitates by centrifuge.
3. MAO can be extracted from a tissue by homogenization with buffer containing protease inhibitor and centrifuge at 10000 – 18000 g for 10-20 min at 4°C. (ref. Biochem. J. (1968) 108: 95.)
4. Collect the supernatant on ice and assay immediately or aliquot and store samples at -80°C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at -80°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -80°C. Avoid repeated freeze-thaw cycles.

Samples should be clear and free of particles or precipitates. Avoid using haemolytic, icteric or lipaemic samples.

REAGENT PREPARATION

- **Standard:** It is recommended to prepare standard immediately prior to every use. DO NOT reuse the diluted standard.
  - **a)** Mix 5 µl of 3% H_2O_2 with 1400 µl distilled water.
  - **b)** Further dilute 5 µl of the diluted H_2O_2 from **a)** with 780 µl distilled water to give a 20 µM H_2O_2 stock.
  - **c)** The distilled water serves as zero standard (0 µM), and the rest of the standard serial dilution can be diluted with distilled water as according to the suggested concentration below: 20 µM, 10 µM, 5 µM and 0 µM.
- **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 p-tyramine, 1 µl of Dye Reagent and 1 µl of HRP Enzyme. Transfer 50 µl of Working Reagent to each sample and control wells. Use 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 each blank wells. Use the prepared Working Reagent within 15 min.
- 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 activity detection:

1. Add 45 µl of each sample in one well and add 5 µl of distilled water as Sample well.
2. Add 45 µl of each sample in another well and add 5 µl of 10 µM MAO-A inhibitor, clorgyline, as Control well.
3. Gently tap the plate to ensure thorough mixing. Incubate for 10 min at room temperature for the inhibitor to block MAO-A activity.
4. Prepare enough Assay Working Reagent for all sample and calibrator wells.
5. Add 50 µl of standards in standard wells.
6. Add 50 µl of Assay Working Reagent to each well.
7. Gently tap the plate to ensure thorough mixing.

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 activity detection:

1. Add 45 µl of each sample in one well and add 5 µl of distilled water as **Sample well.**
2. Add 45 µl of each sample in another well and add 5 µl of 10 µM MAO-B inhibitor, pargyline, as **Control well.**
3. Gently tap the plate to ensure thorough mixing. Incubate for 10 min at room temperature for the inhibitor to block MAO-B activity.
4. Prepare enough Assay Working Reagent for all sample and calibrator wells.
5. Add 50 µl of standards in **standard** wells.
6. Add 50 µl of Assay Working Reagent to each well.
7. Gently tap the plate to ensure thorough mixing.
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 total MAO activity detection:

1. Add 50 µl of each sample in two separate wells.
2. Add 50 µl of standards in **standard** wells.
3. Add 50 µl of **Assay Working Reagent** into one of the sample well **(sample well)** and **standard** wells.
4. Add 50 µl of **Blank Working Reagent** into another sample well **(Blank well).**
5. Gently tap the plate to ensure thorough mixing.
6. Incubation the plate in dark at the room temperature (25°C) for 20 min.

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

For MAO inhibitors screening or characterize inhibitor potency (IC50) (Standards were not necessary)

1. Add 45 µl of each sample (MAO-A or MAO-B purified protein, which is not supplied in this kit) in each wells.
2. Add 5 µl of inhibitor of screening as **test sample** wells.
3. Add 5 µl of 10 µM of MAO-A inhibitor Clorgyline or MAO-B inhibitor Pargyline in another wells can be used as **positive control** wells.
4. Add 5 µl of solvent that the test compounds are dissolved in one well as **no Inhibitor control** well.
5. Add 5 µl of solvent that the test compounds are dissolved in another well as **Blank** well.
6. Gently tap the plate to ensure thorough mixing.
7. Incubate for at least 10 min at room temperature to allow the inhibitor to interact with the enzyme, prior to adding the Working Reagent.
8. Prepare enough **Assay Working Reagent** and **Blank Working Reagent.**
9. Add 50 µl of **Assay Working Reagent** to all **test sample and control** wells.
10. Add 50 µl of **Blank Working Reagent** to **Blank well.**
11. Gently tap the plate to ensure thorough mixing.
12. Incubation the plate in dark at the room temperature (25°C) for 20 min.
13. Read fluorescence intensity at λ ex = 530 nm and λ em = 585 nm.

CALCULATION OF RESULTS

1. Plot H_2O_2 calibration curve and determine its Slope (μM^{-1}). MAO enzyme activity in the sample is calculated as follows:

MAO-A/MAO-B Activity (U/L) =

$(RFU_{Sample} - RFU_{Control}) / (Slope \times t\ (time))$

Total MAO Activity (U/L) =

$(RFU_{Sample} - RFU_{Blank}) / (Slope \times t\ (time))$

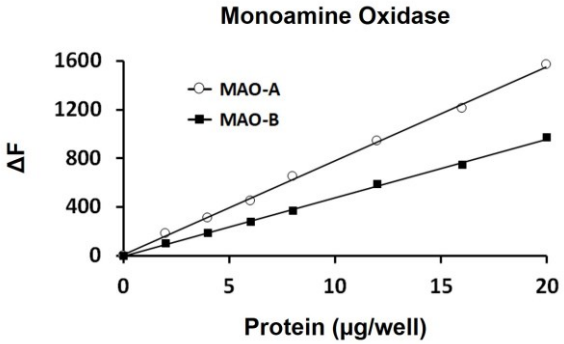
For MAO inhibitors screening (activity %) =

$[(RFU_{Sample} - RFU_{Blank}) / (RFU_{no\ inhibitor\ control} - RFU_{Blank})] * 100\%$

- RFU_{Sample} and $RFU_{Control}$ are the measured fluorescence values of the sample well and sample control well (with inhibitor pargyline or clorgyline).
- $RFU_{no\ inhibitor\ control}$ are the measured fluorescence values of the well with purified enzyme and solvent that the test compounds are dissolved.
- RFU_{Blank} are the measured fluorescence values of the sample blank well with Blank Working Reagent.
- t is the incubation time (20 min).

2. Unit definition: one unit of MAO catalyzes the formation of 1 µmole H_2O_2 per min under the assay conditions.

EXAMPLE OF TYPICAL STANDARD CURVE



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Monoamine Oxidase Activity was 0.01 U/L.