Description

Monoamine Oxidase A (MAO-A) Inhibitor Screening Fluorogenic Assay Kit is designed to measure human MAO-A activity for screening and profiling applications based on the fluorometric detection of H_2O_2, one of the byproducts generated during the oxidative deamination of MAO-A substrates. The assay kit comes in a convenient 96-well format, with enough purified MAO-A enzyme, substrate, HydroRed Probe, HydroRed Developer, and assay buffer for 100 enzyme reactions.

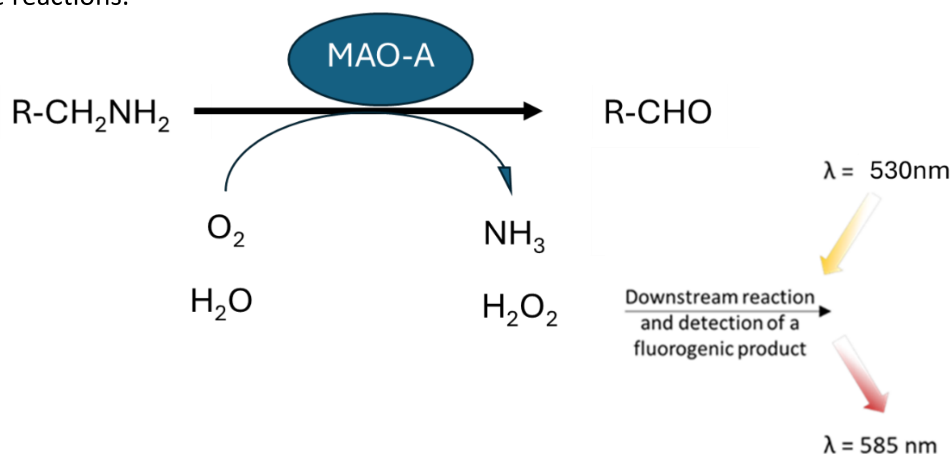

Figure 1: Mechanism of action of Monoamine Oxidase A (MAO-A) Inhibitor Screening Fluorogenic Assay Kit.
Monoamine Oxidase A (MAO-A) catalyzes the oxidative deamination of a primary amine substrate in the presence of oxygen and water. The byproduct hydrogen peroxide (H_2O_2) generated during the MAO-A reaction serves as a substrate for the downstream HydroRed Developer reaction, which oxidizes the HydroRed Probe to produce a strong fluorescence product, resorufin. The resulting fluorescence intensity is directly proportional to MAO-A enzyme activity.

Background

Monoamine oxidase (MAO) is an enzyme on the mitochondrial outer membrane that catalyzes the deamination of biogenic and xenobiotic amines, such as neuroactive serotonin, noradrenaline and dopamine. MAO exists in two isoforms, MAO-A and MAO-B. MAO-A, primarily located in the intestinal tract, the liver, and peripheral adrenergic neurons, regulates mood-related neurotransmitters and is a key target for antidepressants and anxiolytics. Beyond its role in mental health, MAO-A contributes to oxidative stress in cardiac tissue, producing hydrogen peroxide that can damage mitochondria and lead to heart failure. Inhibitors may therefore prove beneficial in the treatment of cardiovascular diseases. High level expression of MAO-A is also linked to prostate cancer, where inhibition reduces proliferation in androgen-sensitive and androgen-resistant cells. Thus, MAO-A inhibitors hold therapeutic potential for depression, anxiety, heart failure, and certain cancers, making them an important focus for drug discovery.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
83904-KC5⁺	MAO-A, His-tag*	5 µg	-80°C
83905-KC4	5x MAO Assay Buffer	4 ml	-20°C
83906-KC120	100x MAO-A Substrate	120 µl	-20°C
83907-KC154	HydroRed Probe	154 µg	-20°C
83908-KC50	HydroRed Developer	50 µl	-20°C
79685	96-well black microplate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Fluorescence plate reader capable of measurement at $\lambda_{ex}530/\lambda_{em}585$ nm.

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle

Enzyme activity is quantified by fluorometric detection of H_2O_2 ($\lambda_{excitation}$ = 530 nm; $\lambda_{emission}$ = 585 nm), a byproduct of oxidative deamination of the MAO-A substrate. The amount of H_2O_2 is directly proportional to MAO-A activity.

Contraindications

- Reagents/substances that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- For inhibition studies the final concentration of DMSO in the assay should not exceed 1%.
- For inhibition studies it is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

- We recommend using Clorgyline (#83953) as an internal control inhibitor. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- If a preincubation step is required, we recommend preincubating the enzyme with the inhibitor prior to adding the substrate for at least 30 minutes.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Reaction

1. Thaw **MAO-A** and **HydroRed Developer** on ice. Briefly spin the tubes to recover their full content.

2. Thaw **5x MAO Assay Buffer** and **MAO-A Substrate** at Room Temperature (RT). Briefly spin the tubes to recover their full content.

3. Prepare 20 ml of **1x Assay Buffer** by diluting **5x MAO Assay Buffer** 5-fold with distilled water.

4. Dilute **MAO-A** to 1.25 ng/µl with 1x Assay Buffer (40 µl/well).

5. Add 40 µl of **diluted MAO-A** to every well.

6. Prepare the **Test Inhibitor** (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   6.1 If the **Test Inhibitor** is water-soluble: Prepare serial dilutions in 1x Assay Buffer, 10-fold more concentrated than the desired final concentrations.

   For the blank, positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   6.2 If the Test activator is soluble in DMSO: Prepare the test activator at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Assay Buffe to keep the concentration of DMSO constant.

   For controls, prepare 10% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

7. Add 5 µl of **Test Inhibitor** to each well labeled “Test Inhibitor”.

8. Add 5 µl of **1x Diluent Solution** to the “Blank” and “Positive Control" wells.

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9. Preincubate the plate at RT for 30 minutes.

10. Dilute **100x MAO-A Substrate** 10-fold with 1x Assay Buffer to prepare **10x MAO-Substrate** (5 µl/well).

11. Add 5 µl of **10x MAO-Substrate** to each well labeled "Positive Control" and "Test Inhibitor".

12. Add 5 µl of 1x Assay Buffer to the "Blank" wells.

13. Incubate the reaction at RT for 20 minutes.

Step 2: Detection

Protect reagents and samples from direct exposure to light.

1. Add 60 µl of the DMSO to the **HydroRed Probe** vial and dissolve the content.

2. Prepare *in the dark* a **Detection Master Mix** (50 µl/well): N wells x (49 µl of 1x Assay Buffer + 0.5 µl of HydroRed Probe + 0.5 µl of HydroRed Developer).

3. Initiate the detection by adding *in the dark* 50 µl of Detection Master Mix to all the wells.

Component	Blank	Positive Control	Test Inhibitor
Diluted MAO-A (1.25 ng/µl)	40 µl	40 µl	40 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Pre-incubate 30 minutes at RT			
10x MAO-A Substrate	-	5 µl	5 µl
1x Assay Buffer	5 µl		
Incubate 20 minutes at RT			
Detection Master Mix	50 µl	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

4. Cover the plate and incubate at RT, protected from light, until you are ready to measure the fluorescence. For this assay, a 5–10 minutes incubation is sufficient. You may also perform kinetic analysis for up to one hour.

   Note: For kinetic analysis use kinetic mode with a recommended kinetic interval of 5 minutes.

5. Read the plate in a fluorescent microplate reader capable of reading λex/λem =530 nm/585 nm (10 nm bandwidth).

6. Calculate results by subtracting the "Blank" value from the other values.

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Example Results

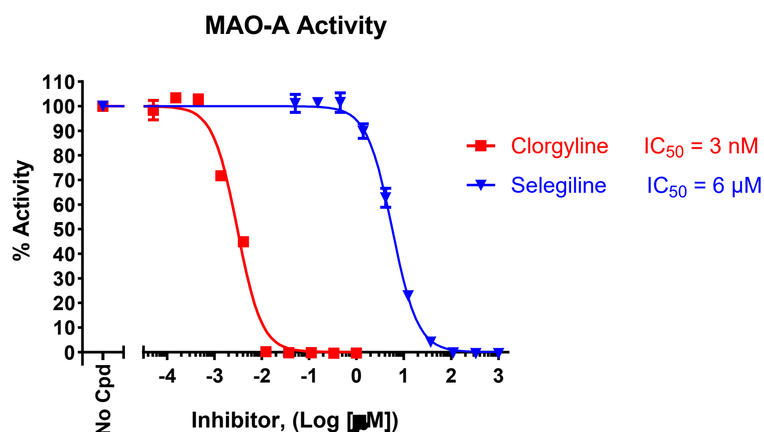

Figure 2: Inhibition of MAO-A by the inhibitors Clorgyline and Selegiline.
MAO-A activity was measured in the presence of increasing concentrations of Clorgyline (#83953) and Selegiline (#83954). The "Blank" value was subtracted from all other values. Results are expressed as the percentage of control (MAO-A activity in the absence of inhibitor, set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
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