Technical Bulletin

Monoamine Oxidase (MAO) Inhibitor Screening Kit

Catalogue number MAK520

Product Description

Monoamine oxidases (MAO, EC 1.4.3.4) are a family of mitochondrial enzymes that catalyze the oxidative deamination of monoamines. Two isoforms of MAO exist, MAO-A and MAO-B, with different inhibitor selectivity and tissue distribution. MAO dysfunction is thought to be responsible for a number of neurological disorders. Unusually high or low levels of MAOs in the body have been associated with depression, schizophrenia, substance abuse, attention deficit disorder, migraines, and irregular sexual maturation. MAO inhibitors are one of the major classes of drug prescribed for the treatment of depression, Parkinson's and Alzheimer's diseases.

Our MAO Inhibitor Screening Assay Kit provides a convenient fluorometric means to screen for MAO enzyme inhibitors. In the assay, MAO reacts with p-tyramine, a substrate for both MAO-A and MAO-B, resulting in the formation of H_2O_2, which is determined by a fluorometric method (λ_{em} = 585 nm/λ_{ex} = 585/530 nm). The assay is simple, sensitive, stable and high-throughput adaptable.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer (pH 7.4) 12 mL
  Catalogue Number MAK520A
- p-Tyramine 120 µL
  Catalogue Number MAK520B
- Pargyline (20 mM) 50 µL
  Catalogue Number MAK520C
- Clorgyline (20 mM) 50 µL
  Catalogue Number MAK520D
- HRP Enzyme 120 µL
  Catalogue Number MAK520E
- Dye Reagent 120 µL
  Catalogue Number MAK520F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescent multiwell plate reader
- Black flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- Purified MAO-A (Catalogue Number M7316 or equivalent)
  Purified MAO-B (Catalogue Number M7441 or equivalent)
- 1.5 mL microcentrifuge tube

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Note: Thiols such as β-mercaptoethanol, dithioerythritol, etc., at concentrations >10 µM interfere with this assay and should be avoided.

Procedure

All Samples and Standards should be run in duplicate.

Note: This procedure is written for 96-well plates.

Sample Preparation

1. Dilute purified MAO-A to 3 U/mL and MAO-B to 6 U/mL using purified H_2O in a 1.5 mL centrifuge tube.
2. Dissolve the test compounds in solvent of choice in a microcentrifuge tube. It is prudent to first test the tolerance of the solvent by the enzyme of choice.

Note: If using DMSO, its concentration in the 5 µL of test compounds added to the reaction should be 10 v/v% or less when screening with human MAO.

Human MAO-A Samples

- Prepare a 1.5-fold dilution of the provided p-Tyramine by adding 80 µL p-Tyramine to 40 µL purified H_2O.

Human MAO-B Samples

- Prepare a 4-fold dilution of the provided p-Tyramine by adding 30 µL p-Tyramine to 90 µL dH_2O.

Note: If another species is being analyzed, we recommend that you experimentally determine the K_m 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 K_m.

MAO Reaction Preparation

1. To determine MAO inhibition, transfer 45 µL of either diluted MAO-A or MAO-B into separate wells. Reserve at least one MAO well for no substrate (Blank), and one without inhibitor test compounds (Control).
2. To the Control and Blank well, add 5 µL of solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 10 v/v% DMSO, add 5 µL 10 v/v% DMSO to these wells.
3. To the remainder of the wells containing MAO-A or MAO-B, add 5 µL of the test compounds.
4. Mix and incubate for 15 min at 25 °C for the inhibitor to block MAO A activity.
5. For a MAO-A positive inhibitor control, dilute the provided 20 mM Clorgyline with purified H_2O to 10 µM. Add 5 µL of 10 µM Clorgyline to the MAO-A Positive Inhibitor Control.
6. For a MAO-B positive inhibitor control, dilute the provided 20 mM Pargyline with purified H_2O to 10 µM. Add 5 µL of 10 µM pargyline to MAO-B Positive Inhibitor Control.

Preparation of Working Reagent

Prepare working reagent as shown as Table 1 for each well.

Table 1.

Reagent	Volume
Assay Buffer	50 µL
1.5-fold diluted p-Tyramine (MAOA) OR 4-fold diluted p-Tyramine (MAO-B)	1 µL
Dye Reagent	1 µL
HRP Enzyme	1 µL

Measurement

1. Transfer 50 µL Working Reagent to all wells.
2. Briefly tap plate to mix.
3. Incubate for 20 min in the dark.
4. Read fluorescence intensity at λ_{ex} = 530 nm and λ_{em} = 585 nm

Results

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

$$\% \text{ MAO Activity} = \frac{RFU_{\text{test cpd}} - RFU_{\text{Blank}}}{RFU_{\text{No inhibitor}} - RFU_{\text{Blank}}} \times 100\%$$

$RFU_{\text{test cpd}}$ = Fluorescence Intensity of Test Compound

RFU_{Blank} = Fluorescence Intensity of Blank (No substrate)

$RFU_{\text{No Inhibitor}}$ = Fluorescence Intensity of Sample with No Inhibitor

Figure 1.
Typical representative curve for MAO-A activity.

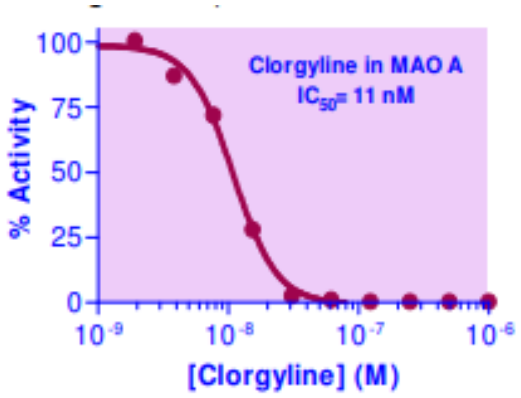
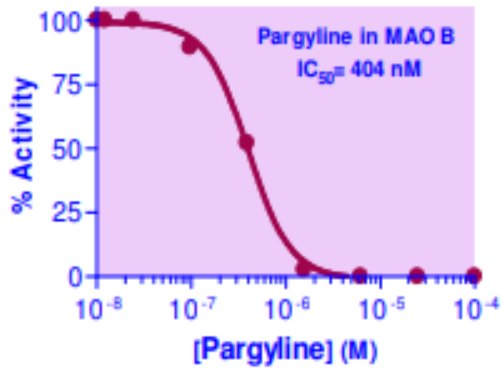


Figure 2.
Typical representative curve for MAO-B activity.



Inhibitor titrations: Human MAO-A and MAO-B were incubated with various concentrations of clorgyline or pargyline respectively. Each concentration of inhibitor contained 10 v/v% DMSO (final 0.5 v/v%). 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.

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