

## Technical Manual

# Monoamine Oxidase Inhibitor Screening Kit

**Catalogue Code: BA0188**

**Pack Size: 100 Tests**

**Research Use Only**

## 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. The Assay Genie MAO Inhibitor Screening Assay Kit provides a convenient fluorimetric 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<sub>2</sub>O<sub>2</sub>, which is determined by a fluorimetric method ( $\lambda_{em/ex}$  = 585/530 nm). The assay is simple, sensitive, stable and high-throughput adaptable.

## KEY FEATURES

**Safe.** Non-radioactive assay.

**Fast.** A 10 min, single reagent, "mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

**Robust and amenable to HTS.** Can be readily automated with HTS liquid handling systems.

## APPLICATIONS

**HTS** for inhibitor screening and evaluation of MAO inhibitors.

## KIT CONTENTS (100 Tests in 96-Well Plate)

<b>Assay Buffer:</b>	12 mL (pH 7.4)	<b><i>p</i>-Tyramine:</b>	120 $\mu$ L
<b>Pargyline:</b>	50 $\mu$ L 20 mM	<b>HRP Enzyme:</b>	120 $\mu$ L
<b>Clorgyline:</b>	50 $\mu$ L 20 mM	<b>Dye Reagent:</b>	120 $\mu$ L

**Storage conditions.** The kit is shipped on ice. Store all components at -20°C. Shelf life: 6 months after receipt.

**Precautions:** Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

## PROCEDURE

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 multi-channel pipettor is recommended.

**Note:** Neither the enzyme MAO-A nor MAO-B is included in the kit.

**Note:** thiols ( $\beta$ -mercaptoethanol, dithioerythritol etc) at > 10  $\mu$ M interfere with this assay and should be avoided in sample preparation.

**Sample Preparation:** Dilute purified MAO-A to 3 U/mL and MAO-B to 6 U/mL using dH<sub>2</sub>O. Dissolve the test compounds in solvent of choice. It is prudent to first test the tolerance of the solvent by the enzyme of choice. If using DMSO, its concentration in the 5  $\mu$ L of test compounds added to the reaction should be 10 v/v% or less when screening with human MAO.

**Reagent Preparation:** Use black flat-bottom plates. Prior to assay, equilibrate all components to room temperature, briefly centrifuge tubes before opening. The Working Reagent should be prepared fresh and used within 15 min.

The following protocol is optimized for human MAO. 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  $\mu$ L reaction is near the  $K_m$ . For human MAO-A, use a 1.5-fold dilution of the

provided p-Tyramine by adding 80  $\mu\text{L}$  p-Tyramine to 40  $\mu\text{L}$  dH<sub>2</sub>O. For human MAO-B, use a 4-fold dilution of the provided p-Tyramine by adding 30  $\mu\text{L}$  p-Tyramine to 90  $\mu\text{L}$  dH<sub>2</sub>O.

## ASSAY

- To determine MAO inhibition, transfer 45  $\mu\text{L}$  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 (Control).
- To the Control and Blank well, add 5  $\mu\text{L}$  of solvent that the test compounds are dissolved in. For example, if the test compounds are dissolved in 10 v/v% DMSO, add 5  $\mu\text{L}$  10 v/v% DMSO to these wells.
- To the remainder of the wells containing MAO-A or MAO-B, add 5  $\mu\text{L}$  of the test compounds. Mix and incubate for 15 min at 25°C for the inhibitor to block MAO A activity.  
For a MAO-A positive inhibitor control, dilute the provided 20 mM clorgyline with dH<sub>2</sub>O to 10  $\mu\text{M}$  (i.e. mix 5  $\mu\text{L}$  20 mM clorgyline with 10 mL dH<sub>2</sub>O). Add 5  $\mu\text{L}$  of 10  $\mu\text{M}$  clorgyline to MAO-A. For a MAO-B positive inhibitor control, dilute the provided 20 mM pargyline with dH<sub>2</sub>O to 10  $\mu\text{M}$  (i.e. mix 5  $\mu\text{L}$  20 mM pargyline with 10 mL dH<sub>2</sub>O). Add 5  $\mu\text{L}$  of 10  $\mu\text{M}$  pargyline to MAO-B.
- Prepare enough Working Reagent for all wells. For each well, mix: 50  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  of either 1.5-fold diluted p-Tyramine (MAO- A) or 4-fold diluted p-Tyramine (MAO-B), 1  $\mu\text{L}$  Dye Reagent and 1  $\mu\text{L}$  HRP Enzyme. Transfer 50  $\mu\text{L}$  Working Reagent to all wells. Briefly tap plate to mix.
- Incubate for 20 min in the dark. Read fluorescence intensity at  $\lambda_{\text{exc}} = 530 \text{ nm}$  and  $\lambda_{\text{em}} = 585 \text{ nm}$ .

## CALCULATION

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

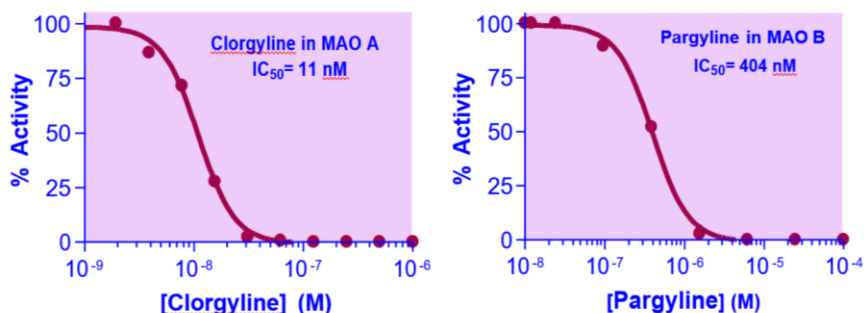
$$\% \text{ Activity} = \frac{\text{RFU}_{\text{Test CPD}} - \text{RFU}_{\text{Blank}}}{\text{RFU}_{\text{No Inhibitor}} - \text{RFU}_{\text{Blank}}} \times 100\%$$

Where the RFU value of the Blank well is MAO without substrate at 20 min.

## MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes black flat-bottom 96-well plate (e.g. Corning Costar).

## EXAMPLE



*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 IC<sub>50</sub> for Clorgyline with 3 U/mL human MAO-A was determined to be 11 nM; while the IC<sub>50</sub> for pargyline with 6 U/mL human MAO-B was determined to be 404 nM.

**Contact Details**  
**Dublin, Ireland**

**Email:** [info@assaygenie.com](mailto:info@assaygenie.com)

**Web:** [www.assaygenie.com](http://www.assaygenie.com)

**Technical Support:** [Techsupport@assaygenie.com](mailto:Techsupport@assaygenie.com)

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