

EnzyChrom™ Monoamine Oxidase Inhibitor Screening Kit (EIMO-100)

Rapid Fluorimetric Screening for Monoamine Oxidase Inhibitors

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.

BioAssay Systems' 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 H₂O₂, 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.

Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Robust and amenable to HTS: can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

HTS for inhibitor screening and evaluation of MAO inhibitors.

KIT CONTENTS

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

ASSAY 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 (β -mercaptoethanol, dithioerythritol etc) at > 10 μ M interfere with this assay and should be avoided in sample preparation.

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.

Sample Preparation: Dilute purified MAO-A to 3 U/mL and MAO-B to 6 U/mL using dH₂O. 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 μ L of test compounds added to the reaction should be 10 v/v% or less when screening with human MAO.

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 μ 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 μ L *p*-Tyramine to 40 μ L dH₂O. For human MAO-B, use a 4-fold dilution of the provided *p*-Tyramine by adding 30 μ L *p*-Tyramine to 90 μ L dH₂O.*

MAO Reaction Preparation:

- To determine MAO inhibition, transfer 45 μ 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 ("No Inhibitor" Control).
- To the No Inhibitor Control and Blank wells, add 5 μ 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 μ L 10 v/v% DMSO to these wells.
- To the remainder of the wells containing MAO-A or MAO-B, add 5 μ L of the test compounds or, if desired, 5 μ L of positive inhibitor control. The MAO-A/MAO-B positive inhibitor controls are prepared by diluting 20 mM clorgyline or pargyline, respectively with dH₂O to 10 μ M (e.g. mix 5 μ L 20 mM inhibitor with 10 mL dH₂O).
Mix and incubate for 15 min at 25°C for the inhibitor to block MAO-A or MAO-B activity.
- Prepare enough Working Reagent by mixing 50 μ L Assay Buffer, 1 μ L of either 1.5-fold diluted *p*-Tyramine (MAO-A) or 4-fold diluted *p*-Tyramine (MAO-B), 1 μ L Dye Reagent and 1 μ L HRP Enzyme for each well (except for the Blank well). Prepare enough Blank Working Reagent by mixing 50 μ L Assay Buffer, 1 μ L dH₂O, 1 μ L Dye Reagent and 1 μ L HRP Enzyme for each Blank well. Transfer 50 μ L Blank Working Reagent to the Blank wells. Transfer 50 μ L Working Reagent to the remaining wells. Briefly tap plate to mix.
- Incubate for 20 min in the dark. Read fluorescence intensity at $\lambda_{exc} = 530$ nm and $\lambda_{em} = 585$ nm.

CALCULATION

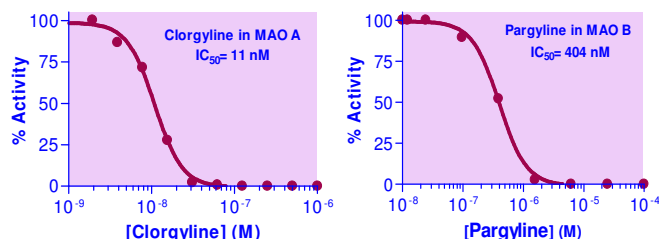
The percent of MAO activity in the presence of a test compound is calculated as follows:

$$\% \text{ Activity} = \left(\frac{\text{RFU}_{\text{Test Cpd}} - \text{RFU}_{\text{Blank}}}{\text{RFU}_{\text{No Inhibitor}} - \text{RFU}_{\text{Blank}}} \right) \times 100\%$$

Where RFU_{Test Cpd}, RFU_{Blank}, and RFU_{No Inhibitor} are the values for the Test Compound, Blank (no substrate), and No Inhibitor wells, respectively.

MATERIALS REQUIRED, BUT NOT PROVIDED

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



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.

LITERATURE

- Ivanovic, I.D. and Majkic-Singh, N. (1988). Determination of platelet monoamine oxidase by new continuous spectrophotometric method. *J Clin Chem Clin Biochem.* 26: 447-51.
- Suzuki, O. et al. (1976). A simple fluorometric assay for type B monoamine oxidase activity in rat tissues. *J. Biochem.* 79: 1297-1299.
- Yamazaki, Mikio, et al. (1987). Monoamine oxidase inhibitors from a fungus, *Emericella navahoensis*. *Chemical and pharmaceutical bulletin* 36.2 (1988): 670-675.

