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 H_2O_2, 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)	***p*-Tyramine:**	120 µL
Pargyline:	50 µL 20 mM	**HRP Enzyme:**	120 µL
Clorgyline:	50 µL 20 mM	**Dye Reagent:**	120 µL

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 µM 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 dH_2O. 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 µL 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 µL reaction is near the K_m. *For human MAO-A, use a 1.5-fold dilution of the provided p-Tyramine by adding 80 µL p-Tyramine to 40 µL dH_2O. For human MAO-B, use a 4-fold dilution of the provided p-Tyramine by adding 30 µL p-Tyramine to 90 µL dH_2O.*

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 (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. 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_2O to 10 µM (i.e. mix 5 µL 20 mM clorygine with 10 mL dH_2O). Add 5 µL of 10 µM clorgyline to MAO-A. For a MAO-B positive inhibitor control, dilute the provided 20 mM pargyline with dH_2O to 10 µM (i.e. mix 5 µL 20 mM clorygine with 10 mL dH_2O). Add 5 µL of 10 µM pargyline to MAO-B.
4. Prepare enough Working Reagent for all wells. For each well, mix: 50 µL Assay Buffer, 1 µL of either 1.5-fold diluted p-Tyramine (MAO-A) or 4-fold diluted p-Tyramine (MAO-B), 1 µL Dye Reagent and 1 µL HRP Enzyme. Transfer 50 µL Working Reagent to all wells. Briefly tap plate to mix.
5. Incubate for 20 min in the dark. Read fluorescence intensity at λ_{exc} = 530 nm and λ_{em} = 585 nm.

CALCULATION

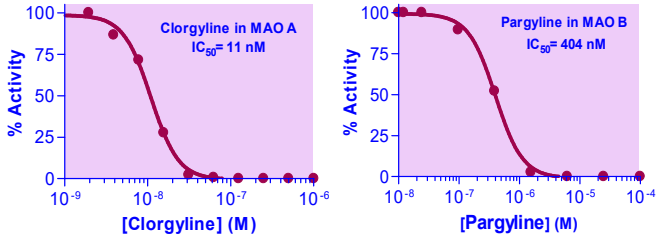
The percent of MAO activity in the presence of a test compound is calculated as follows:

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

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).



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_{50} for Clorgyline with 3 U/mL human MAO-A was determined to be 11 nM; while the IC_{50} for pargyline with 6 U/mL human MAO-B was determined to be 404 nM.

LITERATURE

1. 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.
2. Suzuki, O. et al. (1976). A simple fluorometric assay for type B monoamine oxidase activity in rat tissues. J. Biochem. 79: 1297-1299.
3. Yamazki, Mikio, et al. (1987). Monoamine oxidase inhibitors from a fungus, Emericella navahoensis. Chemical and pharmaceutical bulletin 36.2 (1988): 670-675.