Monoamine Oxidase B (MAO-B) Inhibitor Screening Kit (Fluorometric)

(Catalog # K797-100; 100 assays; Store at -20°C)

I. Introduction:

Monoamine oxidases (MAO, EC 1.4.3.4) are a family of enzymes that can oxidize a wide variety of endogenous primary amines. Two isoforms, MAO-A and MAO-B, have been identified based on their substrate, inhibitor specificity, and tissue localization. MAO-B can oxidize primary amines, but its list of specific substrates (i.e. benzylamine, phenylethylamine) is more limited compared to MAO-A. MAO-B is a mitochondrial-bound enzyme that is ubiquitously expressed throughout the brain and other tissues. It has been investigated in numerous studies including Parkinson's disease, Alzheimer's, and tobacco addiction. Specific MAO-B inhibitors such as selegiline, & rasagiline have been used to treat Parkinson's patients, but their benefits are considered rather modest. BioVision's MAO-B Inhibitor Screening Kit offers a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of MAO-B inhibitors. The assay is based on the fluorometric detection of H_2O_2, one of the byproducts generated during the oxidative deamination of MAO substrate (Tyramine).

II. Applications:

Screening/studying/characterizing MAO-B inhibitors

III. Kit Contents:

Components	K797-100	Cap Code	Part Number
MAO-B Assay Buffer	25 ml	WM	K797-100-1
OxiRed™ Probe (in DMSO)	0.2 ml	Red	K797-100-2A
MAO-B Enzyme (Lyophilized)	1 vial	Purple	K797-100-3
MAO-B Substrate (Lyophilized)	1 vial	Blue	K797-100-4
Developer (Lyophilized)	1 vial	Green	K797-100-5
Inhibitor Control (Selegiline) (Lyophilized)	1 vial	Brown	K797-100-6

IV. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **MAO-B Assay Buffer:** Bring to room temperature before use. Store at -20°C.
- **OxiRed™ Probe**: Bring to room temperature before use. Protect from light & moisture. Store at -20°C. Stable for two months.
- **MAO-B Enzyme:** Reconstitute with 22 µl MAO-B Assay Buffer. Mix well. Aliquot & store at -80°C. Stable for two months.
- **MAO-B Substrate:** Reconstitute with 110 µl ddH_2O. Store at -20°C. Stable for two months.
- **Developer:** Reconstitute with 220 µl MAO-B Assay Buffer. Mix well. Store at -20°C. Stable for two months.
- **Inhibitor Control (Selegiline):** Reconstitute with 250 µl ddH_2O to make a stock solution of 2 mM. Mix well. Make a 10 µM working solution by adding 5 µl of the 2 mM stock solution into 995 µl ddH_2O. Store the stock solution at -20°C. Stable for two months. Inhibitor's working solution can be stored at 4°C to use within 24 hrs.

VI. MAO-B Inhibitor Screening Protocol:

1. **Screening Compounds, Inhibitor Control, and Blank Control Preparations:** Dissolve test inhibitors into proper solvent. Dilute to 10X the desired test concentration with MAO-B Assay Buffer before use. Add 10 µl of test inhibitor (S), working solution of Inhibitor Control and MAO-B Assay Buffer (Enzyme Control; EC) into assigned wells.

   Notes:

   a. Preferred final solvent concentration should not be more than 2% by volume. If solvent exceeds 2%, include a Solvent Control to test the effect of the solvent on enzyme activity.

   b. Optional: To check the possible inhibitory effect of test inhibitors on Developer, prepare a parallel test inhibitor well (TI). Inhibitor Control, Selegiline does not inhibit Developer.

2. **MAO-B Enzyme Solution Preparation:** Dilute the stock solution 5 times by adding 2 µl of MAO-B Stock Solution into 8 µl of MAO-B Assay Buffer. For each well, prepare 50 µl MAO-B Enzyme Solution:

MAO-B Assay Buffer	49 µl
Diluted MAO-B Enzyme	1 µl

   Mix. Add 50 µl/well into wells containing test inhibitors, Inhibitor Control and Enzyme Control. Incubate for 10 min. at 37°C.

   Notes:

   a. Always freshly prepare MAO-B Enzyme working solution. Don't store the enzyme working solution.

   b. To check the possible inhibitory effect of test inhibitors on Developer, replace the 1 µl of diluted MAO-B Enzyme with 1 µl of 10 mM H_2O_2. Mix & add 50 µl to the TI well. Incubate for 10 min. at 37°C.

3. **MAO-B Substrate Solution Preparation:** For each well, prepare 40 µl of MAOB Substrate Solution:

MAO-B Assay Buffer	37 µl
MAO-B Substrate	1 µl
Developer	1 µl
OxiRed™ Probe	1 µl

Mix well and add 40 µl of the MAO-B Substrate Solution into each well. Mix well.

4. **Measurement:** Measure the fluorescence (Ex/Em = 535/587 nm) kinetically at 37°C for 10-40 min. Choose two points (T_1 and T_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).
5. **Calculation:** Calculate the slope for all samples, including Enzyme Control (EC), by dividing the net ΔRFU (RFU_2 -RFU_1) values by the time ΔT (T_2 - T_1). Calculate % Relative Inhibition as follows:

$$\% \textbf{ Relative Inhibition } = \frac{(\textbf{Slope of EC} - \textbf{Slope of S})}{\textbf{Slope of EC}} \textbf{ x 100}$$

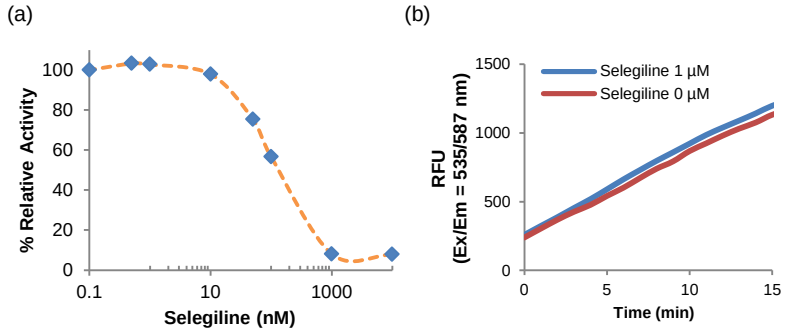

Figure: (a) Inhibition of MAO-B Activity with Selegiline. (b) Selegiline does not inhibit MAO-A activity (an isozyme of MAO-B). Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

Monoamine Oxidase (Total, MAO-A, MAO-B) Activity Fluorometric Assay Kit (K795)
Monoamine Oxidase A (MAO-A) Inhibitor Screening Kit (Fluorometric) (K796)
Rasagiline mesylate (2237)
H_2O_2 (0.88 M) (K266-100-5)

FOR RESEARCH USE ONLY! Not to be used on humans.