

Monoamine Oxidase B (MAO-B) Inhibitor Screening Kit (Fluorometric) (#BN01013)

(Catalog #BN01013; 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. Assay Genie'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 H₂O₂, 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	BN01013	Cap Code	Part Number
MAO-B Assay Buffer	25 ml	WM	BN01013-1
GenieRed Probe (in DMSO)	0.2 ml	Red	BN01013-2A
MAO-B Enzyme (Lyophilized)	1 vial	Purple	BN01013-3
MAO-B Substrate (Lyophilized)	1 vial	Blue	BN01013-4
Developer (Lyophilized)	1 vial	Green	BN01013-5
Inhibitor Control (Selegiline) (Lyophilized)	1 vial	Brown	BN01013-6

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.
- **GenieRed 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 ddH₂O. 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 ddH₂O 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 ddH₂O. 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:

- 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.
- 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:

- Always freshly prepare MAO-B Enzyme working solution. Don't store the enzyme working solution.
- 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 H₂O₂. 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 μ l
MAO-B Substrate	1 μ l
Developer	1 μ l
GenieRed Probe	1 μ l

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

- 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).
- 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:

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

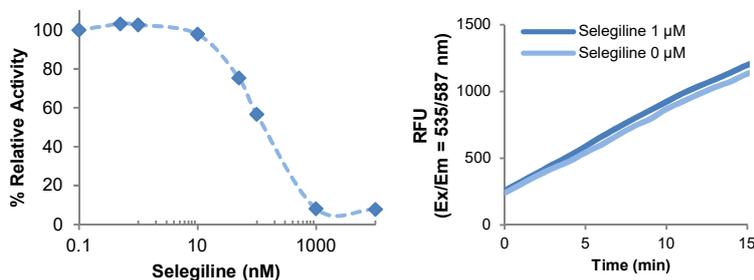


Figure: (a) Inhibition of MAO-B Activity with Selegiline. (b) Selegiline does not inhibit MAO-A activity (an isozyme of MAO-B). Assays were performed following the kit protocol.

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