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Product Information

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

Catalog Number **MAK295**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Monoamine oxidases (MAO, EC 1.4.3.4) are a family of enzymes that 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-A can oxidize primary amines such as serotonin and norepinephrine. MAO-A is a mitochondrial-bound enzyme that is ubiquitously expressed throughout the brain and other tissues. It has been implicated in panic, anxiety, and depression.

Several MAO-A specific inhibitors such as clorgyline, brofaromine, toloxatone, tetrindole, etc. have been used as antidepressants, but their usage has been limited due to side effects. This MAO-A Inhibitor Screening Kit offers a rapid, simple, sensitive, and reliable test suitable for high-throughput screening of MAO-A inhibitors. The assay is based on the fluorometric detection of hydrogen peroxide (H_2O_2), one of the by-products generated during the oxidative deamination of the MAO substrate (Tyramine).

Components

The kit is sufficient for 100 assays in 96 well plates.

MAO-A Assay Buffer Catalog Number MAK295A	25 mL
High Sensitivity Probe (in DMSO) Catalog Number MAK295B	0.2 mL
MAO-A Enzyme Catalog Number MAK295C	1 vial
MAO-A Substrate Catalog Number MAK295D	1 vial
Developer Catalog Number MAK295E	1 vial
Inhibitor Control (Clorgyline) Catalog Number MAK295F	1 vial

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates
- Fluorescence multiwell plate reader
- 10 mM hydrogen peroxide (H_2O_2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

MAO-A Assay Buffer – Bring to room temperature before use. Store at –20 °C.

High Sensitivity Probe – Bring to room temperature before use. Protect from light and moisture. Store at –20 °C. Use within 2 months.

MAO-A Enzyme – Reconstitute with 25 µL of MAO-A Assay Buffer. Mix well. Aliquot and store at –80 °C. Use within 2 months.

MAO-A Substrate – Reconstitute with 110 µL of water. Store at –20 °C. Use within 2 months.

Developer – Reconstitute with 220 µL of MAO-A Assay Buffer. Mix well. Store at –20 °C. Use within 2 months.

Inhibitor Control (Clorgyline) – Reconstitute with 250 µL of water 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 of water. Store the stock solution at –20 °C. Use within 2 months. Inhibitor working solution can be stored at 4 °C to use within 24 hrs.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials prior to opening.

Procedure
Read entire protocol before performing the assay.

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

Note: 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-Clorgyline does not inhibit the Developer.

MAO-A Enzyme Solution Preparation
Dilute the Enzyme stock solution 5 times by adding 2 µL of MAO-A Enzyme Stock Solution into 8 µL of MAO-A Assay Buffer. For each well, prepare 50 µL of MAO-A Enzyme Solution, containing 49 µL of MAO-A Assay Buffer and 1 µL of Diluted MAO-A Enzyme.

Mix. Add 50 µL/well into wells containing test inhibitors, Inhibitor Control, and Enzyme Control. Incubate for 10 minutes at 25 °C.

Note: Always use freshly prepared MAO-A 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-A Enzyme with 1 µL of 10 mM H_2O_2. Mix and add 50 µL to the TI well. Incubate for 10 minutes at 25 °C.

MAO-A Substrate Solution Preparation
For each well, prepare 40 µL of MAO-A Substrate Solution, see Table 1.

Table 1.
Preparation of MAO-A Substrate Solution

Reagent	Samples & Controls
MAO-A Assay Buffer	37 µL
MAO-A Substrate	1 µL
Developer	1 µL
High Sensitivity Probe	1 µL

Mix well and add 40 µL of the MAO-A Substrate Solution into each well. Mix well.

Measurement
Measure the fluorescence kinetically (λ_{ex} = 535 nm/ λ_{em} = 587 nm) at 25 °C for 10–30 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).

Results
Calculations
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:

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	It is recommended to use black plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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