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Monoamine Oxidase Activity (Total MAO/MAO-A/MAO-B) Fluorometric Assay Kit

(Catalog # K795-100; 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. Clonogenic studies have shown that these two isozymes have similar catalytic characteristics, yet their amino acid sequences are different. MAO-A favors Serotonin, Norerpinephrine and Dopamine as substrates, while phenylethylamine and benzylamine are MAO-B preferred substrates. MAO-A and MAO-B are mitochondrial-bound enzymes that are ubiquitously expressed throughout the brain and other tissues. Imbalance of MAOs levels has been associated with schizophrenia, depression, attention deficit and other disorders. MAO-A has been implicated in panic, anxiety and depression, whereas MAO-B defects result in Alzheimer's and Parkinson's diseases. BioVision's Monoamine Oxidase Activity (Total MAO/MAO-A/MAO-B) assay is a sensitive assay to detect total monoamine oxidase activity as well as MAO-A and MAO-B isoenzyme activities separately in the presence of Clorgyline and Selegiline - specific inhibitors for MAO-A and MAO-B, respectively. The assay is based on the fluorometric detection of H_2O_2, one of the byproducts generated during the oxidative deamination of the MAO substrate (Tyramine). The assay can detect as little as 5 µU of MAO enzymatic activity.

II. Application:

- Measurement of Total MAO, MAO-A and MAO-B activities in various biological samples.

III. Sample Type:

- Biological fluids: Serum, plasma
- Animal tissues: Brain, heart, liver, kidney, lung, cell lines (*i.e.* fibroblasts) etc.

IV. Kit Contents:

Components	K795-100	Cap Code	Part Number
MAO Assay Buffer	25 ml	WM	K795-100-1
OxiRed™ Probe (in DMSO)	220 µl	Red	K795-100-2A
MAO Substrate (Lyophilized)	1 vial	Blue	K795-100-3
Developer (Lyophilized)	1 vial	Green	K795-100-4
Positive Control (MAO-A Enzyme) (Lyophilized)	1 vial	Orange	K795-100-5
MAO-A Inhibitor, Clorgyline (Lyophilized)	1 vial	Clear	K795-100-6
MAO-B Inhibitor, Selegiline (Lyophilized)	1 vial	Brown	K795-100-7
H_2O_2 Standard (0.88 M)	100 µl	Yellow	K795-100-8

V. User Supplied Reagents and Equipment:

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

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **MAO Assay Buffer:** Warm Assay Buffer to room temperature before use. Store at 4°C or -20°C.
- **OxiRed™ Probe:** Warm to room temperature before use. Protect from light & moisture. Store at -20°C. Use within two months.
- **MAO Substrate:** Reconstitute with 110 µl ddH_2O to generate a 100 mM solution. Store at -20°C. Stable for two months.
- **Developer:** Reconstitute with 220 µl MAO Assay Buffer. Mix well. Store at -20°C. Stable for two months.
- **Positive Control (MAO-A Enzyme):** Reconstitute with 20 µl MAO Assay Buffer. Mix well. Store at -70°C. Stable for two months.
- **MAO-A Inhibitor, Clorgyline and MAO-B Inhibitor, 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.
- **H_2O_2 Standard:** Bring to room temperature before use. After thawing, store at 4°C. Use within two months.

VII. MAO Activity Assay Protocols:

1. **Sample Preparation:** Biological fluids can be assayed directly. For mammalian tissues, homogenize tissue (1-10 mg) using MAO Assay Buffer (0.1 mg/µl). Centrifuge the homogenate (10000 X g; 10 min.; 4°C). Collect supernatant and keep on ice while in use.
   - To measure total MAO activity, add 1-40 µl of supernatant into desired well(s) in a 96-well plate and adjust the volume to 50 µl/well with MAO Assay Buffer.
   - To measure MAO-A Activity, add 1-40 µl of supernatant and 10 µl of 10 µM Selegiline working solution. Adjust the volume to 50 µl/well with MAO Assay Buffer.
   - To measure MAO-B Activity, add 1-40 µl of supernatant and 10 µl of 10 µM Clorgyline working solution. Adjust the volume to 50 µl/well with MAO Assay Buffer.

   For Positive Control(s), add 1-4 µl Positive Control solution into desired well(s) and adjust the volume to 50 µl/well with MAO Assay Buffer. Incubate the plate for 10 min. at 25°C.

Notes:

a. For unknown samples, we suggest doing a pilot experiment & testing several dilutions to ensure the readings are within the Standard Curve linear range.

b. For samples having H_2O_2 background, prepare parallel sample well(s) as background control.

c. Dilute Positive Control just before use. Don't store the diluted Positive Control.

2. **Standard Curve Preparation:** Dilute H_2O_2 Standard to 10 mM by adding 10 µl of 0.88 M Standard into 870 µl ddH_2O. Further dilute to 0.1 mM by adding 10 µl of 10 mM H_2O_2 Standard into 990 µl ddH_2O. Add 0, 2, 4, 6, 8 and 10 µl of 0.1 mM H_2O_2 Standard into a series of wells in 96-well plate to generate 0, 200, 400, 600, 800, and 1000 pmol/well H_2O_2 Standard. Adjust the volume to 50 µl/well with MAO Assay Buffer.

   Note: Dilute H_2O_2 Standard just before use. Diluted H_2O_2 is unstable.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	* Background Control Mix
MAO Assay Buffer	47 µl	48 µl
Developer	1 µl	1 µl
MAO Substrate	1 µl	----
OxiRed™ Probe	1 µl	1 µl

   Mix well. Add 50 µl of the Reaction Mix to the wells containing Standards, Samples and Positive Control(s). Mix.

   Note: For samples having high background, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm) kinetically at 25°C for 60 min**.**

   Note: Incubation time depends on sample's MAO activity. We recommend measuring fluorescence in a kinetic mode and choosing two time points (T_1 and T_2) in the linear range to calculate the MAO activity of the samples. The Standard Curve can be read in the end point mode (i.e. at the end of incubation time).

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the H_2O_2 Standard Curve. If sample background control reading is high, subtract the sample background control reading from sample reading. Calculate Total MAO, MAO-A and MAO-B activity of the test sample(s): $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the H_2O_2 Standard Curve to get B nmol of H_2O_2 generated by MAOs during the reaction time ($\Delta T = T_2 - T_1$). Calculate total MOA(MAO-T)/MAO-A/MAO-B activity by using the following equations:

Sample MAO-T/MAO-A/MAO-B Activity = B/(ΔT X V) x D = pmol/min/ml = µU/ml

Where: **B** is amount of generated H_2O_2 by MAOs from the Standard Curve (pmol)
ΔT is reaction time (min.)
V is sample volume added into the reaction well (ml)
D is sample dilution factor

MAO's specific activity can also be expressed as µU/mg of protein.
Unit Definition: One unit of MAO activity is the amount of enzyme that generates 1.0 µmol of H_2O_2 per min. at 25°C.

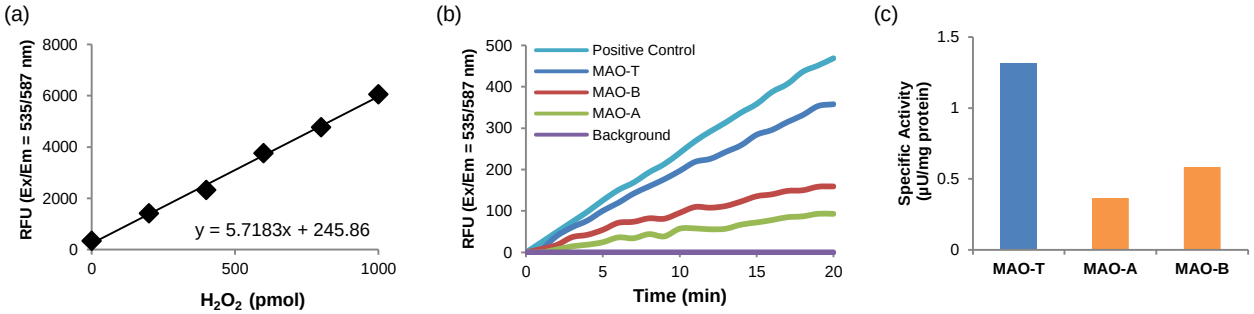

Figure: (a) H_2O_2 Standard Curve. (b) MAO-T, MAO-A and MAO-B activities in mouse brain lysate (2.5 µg) (c) Specific activities of MAO-A and MAO-B. Assays were performed following the kit protocols.

VIII. Related Products:

Monoamine Oxidase A (MAO-A) Inhibitor Screening Kit (K796)
Monoamine Oxidase B (MAO-B) Inhibitor Screening Kit (K797)
Rasagiline mesylate (2237)

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