EnzyChrom™ Monoamine Oxidase Assay Kit (EMAO-100)

Quantitative Determination of Monoamine Oxidase Activity

DESCRIPTION

MONOAMINE OXIDASES (MAO, EC 1.4.3.4) are a family of mitochondrial enzymes that catalyze the oxidative deamination of monoamines. 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.

BioAssay Systems' MAO Assay Kit provides a convenient fluorimetric means to measure MAO enzyme activity. 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.

Sensitive and accurate. As low as 0.01 U/L MAO activity can be quantified.

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

MAO-A/B activity determination in biological samples.

Evaluation and screening for 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

Hydrogen Peroxide: 100 µL 3% H_2O_2

Storage conditions: The kit is shipped on ice. Store all components at -20°C. This product is shipped on ice. 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

Note: (1) thiols (β-mercaptoethanol, dithioerythritol etc) at > 10 µM interfere with this assay and should be avoided in sample preparation.

(2). Samples should be free of particle or precipitates. MAO can be extracted from a tissue by homogenization and differential centrifugation, e.g. *Biochem. J. (1968) 108: 95*. Store sample at -80°C.

(3). Prior to assay, concentrations of protein, inhibitor, substrate and incubation time may need to be established for a given sample.

Use black flat-bottom plates. Prior to assay, bring all components to room temperature, briefly centrifuge tubes before opening.

Dilute the 20 mM inhibitors with H_2O to 10 µM (e.g. mix 5 µL 20 mM inhibitor with 10 mL H_2O).

1. To determine MAO-A activity, use 1 mM p-tyramine substrate and include a control with 0.5 µM MAO-A inhibitor clorgyline.

   Samples: dilute sample in Assay Buffer. Transfer 45 µL of each sample into two separate wells. Add 5 µL H_2O (SAMPLE) and 5 µL 10 µM clorgyline (CONTROL). Mix and incubate for 10 min at room temperature for the inhibitor to block MAO-A activity.

2. Calibrator. Mix 5 µL H_2O_2 with 1400 µL H_2O. Further dilute 5 µL of the resulting H_2O_2 in 780 µL H_2O to give 20 µM H_2O_2. Dilute calibrator with H_2O to give 20, 10, 5 and 0 µM H_2O_2.

   Transfer 50 µL calibrators into separate wells of the assay plate.

3. Prepare enough Working Reagent for all sample and calibrator wells. For each well, mix: 50 µL Assay Buffer, 1 µL p-tyramine, 1 µL Dye Reagent and 1 µL HRP Enzyme. Transfer 50 µL Working Reagent to all wells. Briefly tap plate to mix.

4. Incubate for 20 min in the dark. Read fluorescence intensity at λ_{exc} = 530 nm and λ_{em} = 585 nm.

To measure MAO-B activity, use 1 mM p-tyramine and include a control with 0.5 µM pargyline (MAO-B inhibitor). Procedure is the same as for MAO-A determination.

To screen for MAO inhibitors or characterize inhibitor potency (IC_{50}), mix 5 µL inhibitor with 45 µL sample and incubate for at least 10 min to allow the inhibitor to interact with the enzyme, prior to adding the Working Reagent.

CALCULATION

Plot H_2O_2 calibration curve and determine its Slope (μM^{-1}). MAO enzyme activity in the sample is calculated as

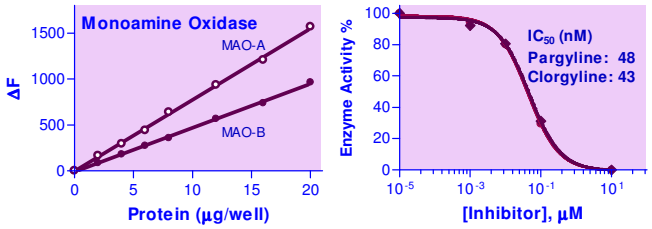
$$\text{MAO Activity} = \frac{RFU_{SAMPLE} - RFU_{CONTROL}}{\text{Slope} \times t} \quad (U/L)$$

where RFU_{SAMPLE} and $RFU_{CONTROL}$ are the measured fluorescence values of the sample and sample control (i.e., in the presence of the respective inhibitor pargyline or clorgyline). *t* is the incubation time (20 min).

Unit definition: one unit of MAO catalyzes the formation of 1 µmole H_2O_2 per min under the assay conditions.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, black flat bottom 96-well plate (e.g. Corning Costar).



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. Youdim, M. B. H. & Tenne, M.(1987). Assay and purification of liver monoamine oxidase. Methods Enzymol. 142, 617 - 626.