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Product Information

Monoamine Oxidase Assay Kit

Catalog Number **MAK136**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Monoamine oxidases (MAO, EC 1.4.3.4) are a family of mitochondrial enzymes that catalyze the oxidative deamination of monoamines. In mammals, MAO consists of two isoenzymes, MAO-A and MAO-B. MAO dysfunction is thought to be responsible for a number of neurological disorders. Unusually high or low levels of MAO 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.

The Monoamine Oxidase Assay Kit provides a convenient fluorimetric means to measure MAO enzyme activity in biological samples. 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 (λ_{ex} = 530/λ_{em} = 585 nm). The assay is simple, sensitive, stable, and high-throughput adaptable. Unit definition: one unit of MAO catalyzes the formation of 1 μmole of H_2O_2 per minute under the assay conditions.

Components

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

Assay Buffer, pH 7.4 Catalog Number MAK136A	12 mL
Pargyline, 20 mM Catalog Number MAK136B	50 μL
Clorgyline, 20 mM Catalog Number MAK136C	50 μL
Hydrogen Peroxide, 3% H_2O_2 Catalog Number MAK136D	100 μL
p-Tyramine Catalog Number MAK136E	120 μL
HRP Enzyme Catalog Number MAK136F	120 μL
Dye Reagent Catalog Number MAK136G	120 μL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

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

Use ultrapure water for the preparation of reagents. Equilibrate all components to room temperature before use. Briefly centrifuge vials before opening. Keep thawed tubes on ice during assay.

Storage/Stability

The kit is shipped on dry ice. If desired, store Assay Buffer and Hydrogen Peroxide at 2–8 °C and all other components at –20 °C.

Procedure

Notes: Thiols (β-mercaptoethanol, dithioerythritol, etc.) >10 μM will interfere with this assay and should be avoided in sample preparation.

Samples should be free of particle or precipitates. MAO can be extracted from a tissue by homogenization and differential centrifugation.[1] Store sample at –80 °C.

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

Inhibitor Preparation

Add 5 μL of 20 mM inhibitor to 10 mL water to generate a 10 μM solution. Clorgyline is an MAO-A inhibitor and pargyline is an MAO-B inhibitor.

Sample Preparation

1. Dilute samples as needed in Assay Buffer. Aliquot 45 μL of each sample into two separate wells, one well will serve as a sample well and one will serve as a sample control well.

2. Add 5 μL of water to the sample well and add 5 μL of the 10 μM inhibitor to the control well. Use clorgyline when assaying for MAO-A and use pargyline when assaying for MAO-B.

3. Mix well using horizontal shaker or by pipetting, and incubate at room temperature for 10 minutes.

For unknown samples, it is suggested to test several sample dilutions.

Hydrogen Peroxide Standards Preparation

Add 5 μL of 3% H_2O_2 to 1,375 μL of water. Further dilute 5 μL of the diluted H_2O_2 solution with 795 μL of water to generate a 20 μM H_2O_2 standard solution. Add 0, 12.5, 25, and 50 μL of 20 μM H_2O_2 standard solution into separate wells of the 96 well plate and bring volume to 50 μL with water generating 0, 5, 10, and 20 μM H_2O_2 standards.

Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 μL of the Master Reaction Mix is required for each sample and standard reaction (well).

Table 1.
Master Reaction Mix

Reagent	Sample and Standards
Assay Buffer	50 μL
p-Tyramine	1 μL
HRP Enzyme	1 μL
Dye Reagent	1 μL

2. Add 50 μL of the Master Reaction Mix to each of the sample, sample control, and standard wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction 20 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the fluorescence (FLU) of the samples and standards at λ_{ex} = 530/λ_{em} = 585.

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

Results

Plot H_2O_2 standard curve and determine the slope (μM^{-1}).

Note: A new standard curve must be set up each time the assay is run.

MAO enzyme activity in the sample is calculated as:

$$\text{MAO Activity (units/L)} = \frac{FLU_{sample} - FLU_{control}}{\text{Slope} \times t}$$

FLU_{sample} = Absorbance measured in unknown sample
$FLU_{control}$ = Absorbance measured in sample control (sample in presence of inhibitor)
Slope = Determined from calibration curve (μM^{-1})
t = incubation time (i.e., 20 minutes)

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

Reference

Biochem. J., **108**, 95 (1968).

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	For fluorescence assays, use black plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use 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 Master 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 calibrator curve	Pipetting errors in preparation of calibrator	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Calibrator stock is at incorrect concentration	Refer to the calibrator 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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