Fluorometric Monoamine Oxidase Assay Kit

Introduction

Monoamine oxidases (MAOs) are flavoproteins mainly distributed in the outer mitochondrial membrane that catalyze the oxidation of amine substrates to produce hydrogen peroxide and ammonia. MAOs are divided into two isoenzymes, MAO-A and MAO-B, and although they share up to 70% sequence similarity, there are significant differences in cell distribution, substrate specificity, and function. MAO-A is mainly distributed in catecholaminergic neurons; Whereas, MAO-B is mainly found in astrocytes and acts on substrates such as dopamine. Studies have shown that the abnormal activation of MAOs is closely related to a variety of neuropsychiatric diseases, such as Alzheimer's disease and Parkinson's disease.

Fluorometric Monoamine Oxidase Assay Kit can be used to rapidly detect the MAOs activity. The detection principle is based on the hydrogen peroxide, a by-product of MAO catalyzed amine substrates such as tyramine. In the presence of HRP, Hydrogen Peroxide reacts with a specific fluorescent probe, Amplex Red, to generate a highly fluorescent resorufin. The fluorescence intensity is proportional to the activity of MAOs. At the same time, the kit provides MAO-A and MAO-B inhibitors, which can detect the total MAO activity as well as MAO-A and MAO-B isoenzyme activities.

Components and Storage

Components \ Size	100 Tests	Storage
Amplex Red Reagent	5 vials	-20°C away from light
DMSO	1.3 mL	-20°C
Horseradish Peroxidase	1 vial	-20°C away from light
Hydrogen Peroxide	500 µL	-20°C
5× Reaction Buffer	28 mL	-20°C
p-Tyramine Hydrochloride	1 vial	-20°C
Clorgyline Hydrochloride	1 vial	-20°C
Selegiline Hydrochloride	1 vial	-20°C
Resorufin	1 vial	-20°C away from light
Shipping: Blue ice	Shelf life: 1 year	

Protocol

1. Preparation before the experiment:

1) Amplex Red (20 mM) Preparation: Amplex Red (20 mM) is made by adding 200 μL of DMSO to 1 vial of Amplex Red Reagent and dissolving thoroughly. 1 vial of Amplex Red (20 mM) is sufficient for 100 tests. Prepared Amplex Red (20 mM) needs to be stored at -20°C protected from light.

2) 1× Reaction Buffer Preparation: Dilute an appropriate amount of 5× Reaction Buffer to 1× Reaction Buffer with ddH_2O. For example, dilute 5 mL of 5× Reaction Buffer in 20 mL of ddH_2O.

3) Horseradish Peroxidase (200 U/mL) Preparation: Dissolve 1 vial of Horseradish Peroxidase in 1 mL of 1× Reaction Buffer to make the Horseradish Peroxidase (200 U/mL). Unused Horseradish Peroxidase (200 U/mL) should be aliquoted and stored at -20°C in the dark.

4) Hydrogen Peroxide (20 mM) Preparation: Dilute the appropriate amount of Hydrogen Peroxide with 1× Reaction Buffer to prepare Hydrogen Peroxide (20 mM). For example, dilute 23 μL of Hydrogen Peroxide in 977 μL of 1× Reaction Buffer and mix gently. Hydrogen Peroxide (20 mM) is unstable and should to be prepared freshly every time.

5) Preparation of p-Tyramine Hydrochloride (100 mM): Dissolve 1 vial of p-Tyramine Hydrochloride with 1.2 mL of ddH_2O to make p-Tyramine Hydrochloride (100 mM). Prepared p-Tyramine Hydrochloride (100 mM) needs to be stored at -20°C.

***Note:** p-Tyramine Hydrochloride is a substrate for MAO-A and MAO-B.

6) MAO inhibitor (0.5 mM) preparation: Dissolve 1 vial of Clorgyline Hydrochloride with 1 mL ddH_2O. Dissolve 1 vial of Selegiline Hydrochloride with 1 mL ddH_2O. The prepared two MAO inhibitors need to be stored at -20°C.

***Note:** Clorgyline Hydrochloride is a MAO-A inhibitor and Selegiline Hydrochloride is a MAO-B inhibitor.

7) (Optional) Resorufin (2 mM) preparation: Dissolve 1 vial of Resorufin with 1 mL of ddH_2O to obtain Resorufin (2 mM). Prepared Resorufin (2 mM) needs to be stored at -20°C in the dark.

2. Sample Preparation:

1) Serum or plasma can be used directly.

2) For cells or tissues, collect 1×10^6-1×10^7 cells or 1-10 mg of tissue, and add 1× Reaction Buffer (e.g., 10-100 μL) for homogenization. Then centrifuge at 10,000 g for 10 min at 4°C, collect supernatant and place on ice for later use.

***Note:**

a) It is better to use a Protease Inhibitor Cocktail, such as Protease Inhibitor Cocktail (EDTA-Free, 100X in DMSO) (Cat. No. K1007).

b) If the reading of samples is low when detecting, you can try replacing the 1× Reaction Buffer with a suitable lysis buffer.

c) Try to use fresh samples for experiments.

3. Hydrogen Peroxide Standard Preparation:

Hydrogen Peroxide is unstable, and the standards need to be used as quickly as soon. Generally, it can be prepared just before the MAO activity test.

1) Dilute an appropriate amount of Hydrogen Peroxide (20 mM) with 1× Reaction Buffer to make Hydrogen Peroxide (100 μM).

2) Refer to the table below to prepare different concentrations of Hydrogen Peroxide standards in 96-well plates. 100 μL of standard is required per well, and it is recommended to prepare at least two parallel wells per set.

Standard #	100 μM Resorufin Standard (μL)	1× Reaction Buffer (μL)	Hydrogen Peroxide (pmol/well)
1	0	100	0
2	4	96	200
3	8	92	400
4	12	88	600
5	16	84	800
6	20	80	1000

4. (Optional) Resorufin Standard Curve Preparation: Serial dilution of the appropriate amount of Resorufin (2 mM) with 1× Reaction Buffer yields a series of Resorufin standards at concentrations between 0-20 μM. Transfer 200 μL of the standards to the 96-well. Detect the fluorescence signal immediately (Ex/Em=535/587 nm).

5. MAO activity assay:

1) Take 1-100 μL of the sample and adjust the volume to 100 μL with 1× Reaction Buffer. It is recommended to prepare at least two parallel wells per sample.

2) MAO inhibitor treatment (optional): This step can be skipped if total MAO-T activity is measured. To measure MAO-A activity, use the MAO-B inhibitor Selegiline Hydrochloride. To measure MAO-B activity, use the MAO-A inhibitor Clorgyline Hydrochloride. For example, add 0.2 μL of MAO inhibitor to 100 μL of sample for a certain period (eg, 30 min) at a final concentration of 1 μM in the system.

***Note:** The treatment time and concentration of MAO inhibitors can be optimized based on the specific experiment.

3) Transfer the samples to 96-well plates containing different concentrations of Hydrogen Peroxide standard. Each well needs 100 μL.

4) Preparation of the reaction mix: Refer to the following table to prepare the reaction mix. The final concentrations of Amplex Red, Horseradish Peroxidase and p-Tyramine Hydrochloride are 400 μM, 2 U/mL, and 2 mM, respectively.100 μL of reaction mix is required per well.

	Reaction mix
Amplex Red (20 mM)	200 μL

Horseradish Peroxidase (200 U/mL)	100 μL
p-Tyramine Hydrochloride (100 mM)	200 μL
1× Reaction Buffer	9.5 mL
Total volume	10 mL

5) Add 100 μL of reaction mix to each well and mix well.

6) Detect the fluorescence signal (Ex/Em=535/587 nm), it is recommended to directly detect the kinetic curve for 20-60 min.

6. Data analysis:

1) Subtract the background value of standard #1 from all standard readings, and then plot the standard curve.

2) To calculate the MAOs activity in the sample:

$$\Delta F = (F(T2) - F(T1))$$

Note: T1 and T2 are selected at different time points, and F is the fluorescence signal value detected.

Apply ΔF to the standard curve to get A pmol of Hydrogen Peroxide in the sample during the reaction time (ΔT=T2-T1).

$$\text{MAO-T/MAO-A/MAO-B Activity} = A \div \Delta T \div V \times B = \text{pmol/min/mL} = \mu\text{U/mL}$$

Note: V is the volume of sample added per well, mL; B is the dilution factor, if the sample is not diluted, B = 1

U definition: Under this condition, the amount of enzyme required to catalyze the substrate to produce 1.0 μmol of Hydrogen Peroxide per minute is one activity unit.

Note

1. Before the formal experiment, it is recommended to perform several dilutions of the sample to ensure the readings are within the standard value range.

2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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