MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Monoamine Oxidase (MAO) Assay Kit

Product Number: BCK0144

Shipping and Storage

1. Reagent 3 store at 4°C and protected from light; Store all other reagents at 4°C.
2. Validity period: 3 months.

Component

Component	96T
Extraction Solution 1	100mL× 1 bottle
Extraction Solution 2	100mL× 1 bottle
Extraction Solution 3	100mL× 1 bottle
Reagent 1	3mL× 1 bottle
Reagent 2	11mL× 1 bottle
Reagent 3	2mL× 1 vials

1. Reagent 1: Ensure powder settles to the bottom before opening (manual flicking is acceptable). Reconstitute each vial with an additional 1.1mL distilled water. The reconstituted reagent can be aliquoted and stored at −20℃.
2. Reagent 3: Ensure powder settles to the bottom before opening (manual flicking is acceptable). Add 3.9mL distilled water for reconstitution. Aliquot unused reagent and store at −20℃. Avoid repeated freeze-thaw cycles and use within three days.

Description

Monoamine oxidase (MAO, EC 1.4.3.4) catalyzes the oxidative deamination of monoamines. MAO is located on the outer mitochondrial membrane of cells. It is widely distributed in various organs of vertebrates, especially secretory glands, brain and liver. It is also found in invertebrates and plant tissues such as bean sprouts, where it catalyzes monoamine metabolism at relatively low levels.

Monoamine oxidase (MAO) catalyzes the deamination of monoamine substrates to form corresponding aldehydes and hydrogen peroxide. The resulting hydrogen peroxide reacts with 4-aminoantipyrine and other reagents to produce a colored substance with maximum absorbance at 510nm. MAO enzyme activity is determined by measuring the change in absorbance at 510nm.

Required Laboratory Equipment

Mortar (homogenizer), ice box (ice maker), benchtop centrifuge, adjustable pipettor, water bath (oven, incubator, metal bath), 96-well plate, centrifuge tube, microplate reader, distilled water (deionized water or ultrapure water is acceptable).

Sample Assay

It is recommended to run a pilot test with 1-3 representative samples of distinct groups/types to get familiar with the workflow. Adjust sample loading concentration according to pilot results to avoid unnecessary consumption of samples and reagents.

1. Sample Extraction

1.1. Tissue Samples

Weigh approximately 0.1 g of sample, add 1 mL of pre-cooled Extraction Solution I (4°C), and fully homogenize on ice. Centrifuge at 1000 g, 4°C for 10 min and discard the pellet. Transfer the supernatant to another pre-cooled centrifuge tube, centrifuge at 10000 g, 4°C for 10 min, discard the supernatant and retain the pellet. Add 1 mL of pre-cooled Extraction Solution II (4°C) to the pellet, vortex thoroughly, then centrifuge at 10000 g, 4°C for 15 min. Remove the supernatant completely and keep the pellet. Add 1 mL of pre-cooled Extraction Solution III (4°C) to the pellet, vortex thoroughly, keep on ice as the test sample (can be directly used for protein concentration determination).

1.2. Liquid samples: Test directly. If turbid, centrifuge and collect the supernatant for testing.

1.3. Bacterial / Cell Samples

Collect bacteria or cells into a centrifuge tube, centrifuge and discard the supernatant. Take 5 million bacteria or cells, add 1mL of pre-cooled Extraction Solution I (4°C), and fully homogenize on ice. Centrifuge at 1000 g, 4°C for 10 min and discard the pellet. Transfer the supernatant to another pre-cooled centrifuge tube, centrifuge at 10000 g, 4°C for 10 min, discard the supernatant and retain the pellet. Add 1mL of pre-cooled Extraction Solution II (4°C) to the pellet, vortex thoroughly, then centrifuge at 10000 g, 4°C for 15 min. Remove the supernatant completely and keep the pellet. Add 1mL of pre-cooled Extraction Solution III (4°C) to the pellet, and sonicate bacteria or cells on ice (power 200 W, sonicate for 3 s, interval 10 s, repeat 30 times). Centrifuge at 12000 rpm, 4°C for 10min, collect the supernatant and keep it on ice for testing.

Note: For larger sample input, perform extraction at ratio: cell count ($\times 10^4$ cells) : Extraction Solution (mL) = 500~ 1000 :1. Cell lysis using commercial lysis buffer is also acceptable; follow the lysis-buffer manufacturer's instructions.

2. Protocol

2.1. Warm up the microplate reader for 30 min (waiting for the instrument to complete self-test is also acceptable), and set the wavelength to 510nm.

2.2. Thaw all reagents to room temperature (25°C).

2.3. Add sequentially into the 96-well plate:

Component	Assay tube
Sample	40μL
Reagent 1	30μL
Reagent2	110μL
Reagent 3	20μL
Mix thoroughly. Incubate at 37°C. Immediately read absorbance A1 at 510nm, then read A2 after 60 min. ΔA = A2 − A1.	

Note: If the ΔA value is low, increase the sample volume V_1 (e.g., up to 80μL, with a corresponding reduction of Reagent II), or extend the reaction time T (e.g., to 2 h or longer). Substitute the revised sample loading volume V_1 and reaction time T into the calculation formula for recalculation.

Calculation of Results

1. Calculated based on sample mass:

Enzyme activity definition: One unit of enzyme activity is defined as a change of 0.001 in absorbance at 510nm per gram of tissue per minute in the reaction system.

MAO activity (ΔOD510 /min/g fresh weight) = ΔA ÷ (W × V1 ÷ V) ÷ 0.001 ÷ T = 416.7 × ΔA ÷ W

2. Calculated based on the protein concentration of the sample:

Enzyme activity definition: One unit of enzyme activity is defined as a change of 0.001 in absorbance at 510 nm per milligram of tissue protein per hour in the reaction system.

MAO (ΔOD510 /min/mg prot) = ΔA ÷ (V1 × Cpr) ÷ 0.001 ÷ T = 416.7 × ΔA ÷ Cpr

3. Calculated based on the volume of the liquid sample:

Enzyme activity definition: One unit of enzyme activity is defined as a change of 0.001 in absorbance at 510 nm per milliliter of liquid sample per minute in the reaction system.

MAO activity (ΔOD510 /min/mL) = ΔA ÷ V1 ÷ 0.001 ÷ T = 416.7 × ΔA

4. Calculated based on bacterial or cell count:

Enzyme activity definition: One unit of enzyme activity is defined as a change of 0.001 in absorbance at 510 nm per gram of tissue per minute in the reaction system.

MAO activity (ΔOD510 /min/10^4 cell) = ΔA ÷ (500 × V1 ÷ V) ÷ 0.001 ÷ T = 416.7 × ΔA ÷ 500

Parameter explanations:

V — Volume of extraction solution added, 1mL;

V1 — Volume of sample added, 0.04mL;

W — Sample weight, g;

T — Reaction time, 60 min;

500 — Total number of bacteria or cells, 10^4;

Cpr — Protein concentration of sample, mg/mL; The BCA protein assay kit from our company is recommended.