Monoamine Oxidase（MAO）Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: BC0015

Size:100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent Name	Size	Storage
Reagent I	Liquid 75 mL×1	2-8℃
Reagent II	Liquid 3.5 mL×1	2-8℃
Reagent III A	Liquid 1.5 mL×1	2-8℃
Reagent III B	Liquid 6 mL×1	2-8℃
Reagent IV	Liquid 0.35 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Preparation of solutions:

1. Reagent III: before use, mix volume of reagent III A: reagent III B= 12 μL: 48 μL (60 μL, 1T) to prepare reagent III according to the sample number.
2. Reagent IV: Liquid is placed in the EP tube in the reagent bottle and should be centrifuged before use. Before use, add 6.65mL of distilled water, mix well. Store the inexhaustible reagents at 2-8℃ for 2 weeks.
3. Standard: 1mg/mL nitrogen standard.
4. 10μg/mL nitrogen standard: Pipette 10μL of 1mg/mL ammonia standard, add 990μL of distilled water and mix well to prepare 10μg/mL Nitrogen Standard.

Product Description:

Monoamine oxidase (EC1.4.3.4) includes MAO-A and MAO-B. It is a flavin protein that binds to the outer membrane of mitochondria and catalyzes the oxidative deamination of neurotransmitters and bioamines. Monoamine oxidase is related to the aging of the body and is considered to be a sign of aging. It mainly exists in various organs of vertebrates, especially secretion glands, brain and liver. It also catalyzes the metabolism of monoamines in invertebrates, bean buds and other plants, with a low content, and has important physiological functions.

MAO catalyzes the deamination of monoamine substrates, and the NH_4^+ produced is measured using the indophenol blue colorimetric method. The product had a characteristic absorption peak at 630 nm, and the MAO enzyme activity could be calculated by measuring the absorbance at 630 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Operation procedure:

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue: according to tissue weight (g): Reagent I (mL) is 1:5~10 to extract. Add 1 mL of Reagent I to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.
2. Bacteria or cells: Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. According to bacteria or cells (10^4): Reagent I (mL) is 5~10:1 to extract. It is suggested to add 1 mL of Reagent I to 5 million of bacteria or cells. Use ultrasonication to split bacteria and cells (placed on ice, ultrasonic power 200W, working time 3 seconds, interval 10 seconds, repeat for 3mins). Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.
3. Serum (plasma) or liquid samples: Detect directly

II. Determination procedure:

1. Preheat visible spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 630 nm, set spectrophotometer to zero with distilled water.
2. Add the following reagents successively into 1.5ml quartz cuvette:

Reagent(μL)	Test Tube (T)	Control Tube (C)	Standard Tube (S)	Blank Tube (B)
Sample	30	-	-	-
Standard	-	-	30	-
Stilled Water	-	-	-	30
Reagent I	120	120	120	120
Reagent II	30	30	30	30
Mix well and react at 37℃ for 20min		-		
Reagent III	60	60	60	60
Reagent IV	60	60	60	60
Sample	-	30	-	-
Mix well and react at RT for 20min. Absorb 200μL to measure the absorbance at 630nm, record as A_T, A_C, A_S, A_B. Calculate $\Delta A_T=A_T-A_C$,$\Delta A_S=A_S-A_B$.The standard tube and blank tube only needs to be measured 1-2 times.				

III. Calculation:

1. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produced 1 μg of ammonium nitrogen (NH_3-N) in the reaction system per minute every milligram protein.

MAO activity (U/mg prot) =$\Delta A_T \div (\Delta A_S \div C_S) \times V_E \div (Cpr \times V_E) \div T \times F = 0.5 \times \Delta A_T \div \Delta A_S \div Cpr \times F$

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produced 1 μg of ammonium nitrogen (NH_3-N) in the reaction system per minute every gram tissue.

MAO activity (U/g weight) =$\Delta A_T \div (\Delta A_S \div C_S) \times V_E \div W \div T \times F = 0.5 \times \Delta A_T \div \Delta A_S \div W \times F$

3. Cells or bacteria

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produced 1 μg of ammonium nitrogen (NH_3-N) in the reaction system per minute every 10^6 cells or bacteria.

MAO activity (U/10^6 cell) =$\Delta A_T \div (\Delta A_S \div C_S) \times V_E \div N \div T \times F = 0.5 \times \Delta A_T \div \Delta A_S \div N \times F$

4. Serum/ plasma or liquid samples

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produced 1 μg of ammonium nitrogen (NH_3-N) in the reaction system per minute every milliliter liquid.

MAO activity (U/mL)= $\Delta A_T \div (\Delta A_S \div C_S) \times V_S \div V_S \div T \times F = 0.5 \times \Delta A_T \div \Delta A_S \times F$

C_S: Concentration of standard,10μg/mL;

V_S: Sample volume, 0.03 mL;

V_E: Reagent I volume, 1 mL;

Cpr: Sample protein concentration, mg/mL;

T: Reaction time, 20 minutes;

W: Sample weight, g;

N: the number of cells, count by 10^6;

F: dilution factor.

References:

[1] H. Soep.The determination of monoamine oxidase activity : Pure and Applied Chemistry[J].Analytical Chemistry, 2009, 45(1):118-24.DOI:10.1021/ac60323a027

Related Products：

BC1280/BC1285 Diamine Oxidase (DAO) Activity Assay Kit

BC1550/BC1555 Glutamic-pyruvic Transaminase (GPT) Activity Assay Kit

BC1560/BC1565 Glutamic-oxalacetic Transaminase (GOT) Activity Assay Kit