Monoamine Oxidase Assay Kit

(Cat/No.:BC159 Size:50T/48S)

1. Assay significance

Monoamine oxidase (MAO) widely exists in vivo, it is abundant in liver, kidney and brain. In tissue, MAO mainly exists on mitochondria (tightly combined with membrane), minority of MAO exists in cytoplasmic soluble ingredients. Blood serum MAO is soluble, it is different from mitochondrion MAO but similar with connective tissue MAO. MAO can promote connective maturation, MAO participates in bridging step of in collagen formation to combine collagen & elastin.

2. Assay principleUltraviolet Colorimetric Method

Under effect of MAO, benzylamine converts to benzylaldehyde which can be extracted by cyclohexane. It is able to measure MAO activity by measuring OD values at 242nm.

3. Composition & Preparation (The kit is valid for 6 months)

Reagent 1: Liquid 20ml×1 bottle, can be stored at 2~8℃away from light for 3 months.

Reagent 2: Liquid 100ml×2 bottles, can be stored at 2~8℃for 6 months.

Reagent 3: Liquid 20ml×1 bottle, can be stored at 2~8℃ for 3 months. Note: This reagent is irritative to skin.

Reagent 4: Liquid 100ml×2 bottles, can be stored at 2~8℃away from light for 3 months.

4. Operation Procedure

（1）Sample pretreatment

① **Blood serum (or plasma):** Can be measured directly.

② **Tissue:** Weigh tissue sample accurately, add 9 times volume physiological saline to prepare 10% homogenate, centrifugate at 2000rpm for 10 minutes, take supernatant to measure MAO activity and protein concentration.

（2）Operation table:

	Blank	Assay
Distilled water (ml)	a*	
Sample (ml)		a*
Reagent 1 (ml)	0.3	0.3
Reagent 2 (ml)	3	3
Mix sufficiently, place in 37℃water bath for 3 hours		
Reagent 3 (ml)	0.3	0.3
Reagent 4 (ml)	3	3
Mix sufficiently, extract contiguously for 2 minutes, centrifugate at 3000~3500rpm for 10 minutes, transfer supernatant in quartz cuvettes of 1cm light path, measure OD values of all tubes at 242nm (adjust zero by blank tube).		

a* is sampling volume**.** Referenced sampling volume:

● 10% brain tissue homogenate: 500μl ● Blood serum: 200～300μl

***** Please wash cuvettes with double distilled water at first, then rinse them with dehydrated alcohol, open-air drying for use.

Note: "extract contiguously" means mixing by vortex contiguously,.

Please use glass test tubes in extraction step, do not use plastic test tube instead.

5. Calculation

(1) Tissue MAO assay:

① **Definition:** React at 37℃, 0.01 OD value increasing per hour per mg protein is considered as 1 MAO activity unit (U).

② **Formula:**

$$\text{MAO activity (U/mgprot)} = \frac{OD_{Sample}}{0.01} \div 3 \text{ hours} \div \frac{\text{Sampling protein amount in mg}}{(\text{sampling volume} \times \text{protein concentration in sample})}$$

③ **Example:**

Take 500μl 10% mouse brain tissue homogenate to measure MAO activity, in results, OD_{Sample} is 0.236, protein concentration in 10% homogenate is 1.6mgprot/ml, calculate as follows:

$$\text{MAO activity (U/mgprot)} = \frac{OD_{Sample}}{0.01} \div 3 \text{ hours} \div \frac{\text{Sampling protein amount in mg}}{\text{(sampling volume} \times \text{protein concentration in sample)}}$$

$$= \frac{0.236}{0.01} \div 3 \div (0.5 \times 1.6)$$

$$= 9.84\ U/mgprot$$

(2) Blood serum (or plasma) MAO assay:

① **Definition:** React at 37℃, 0.01 OD value increasing per hour per ml blood serum (or plasma) is considered as 1 MAO activity unit (U).

② **Formula:**

$$\text{MAO activity (U/ml)} = \frac{OD_{Sample}}{0.01} \div 3 \text{ hours} \div \text{Sampling volume (ml)}$$

③ **Example:**

Take 200μl blood serum to measure MAO activity, in results, OD_{Sample} is 0.080, calculate as follows:

$$\text{MAO activity (U/ml)} = \frac{OD_{Sample}}{0.01} \div 3 \text{ hours} \div \text{Sampling volume (ml)}$$

$$= \frac{0.080}{0.01} \div 3 \div (0.2)$$

$$= 13.33\ U/ml$$

6. Announcements

① After add Reagent 4, total solution volume becomes quite large, so it is suggested to seal test tube by handi-wrap, stop up opening by your finger during extraction by vortex. Centrifugation speed should be ≥3500rpm in order to gradate completely.

② This kit can be used for laboratory research only.