Monoamine Oxidase

Microplate Assay Kit

User Manual

Catalog # CAK1102

(Version 1.2D)

Detection and Quantification of Monoamine Oxidase (MAO) Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Monoamine oxidases (MAO, EC 1.4.3.4) are a family of enzymes that can oxidize a wide variety of endogenous primary amines. Two isoforms, MAO-A and MAO-B, have been identified based on their substrate, inhibitor specificity and tissue localization. Clonogenic studies have shown that these two isozymes have similar catalytic characteristics, yet their amino acid sequences are different. MAO-A favors Serotonin, Norerpinephrine and Dopamine as substrates, while phenylethylamine and benzylamine are MAO-B preferred substrates. MAO-A and MAO-B are mitochondrial-bound enzymes that are ubiquitously expressed throughout the brain and other tissues. Imbalance of MAOs levels has been associated with schizophrenia, depression, attention deficit and other disorders. MAO-A has been implicated in panic, anxiety and depression, whereas MAO-B defects result in Alzheimer's and Parkinson's diseases.

The assay is initiated with the enzymatic catalysis of the substrate by MAO. The enzyme catalysed reaction products can be measured at a colorimetric readout at 490 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Diluent	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard (10 mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 15 ml Diluent to dissolve before use.

Dye Reagent: add 4 ml Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 490 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml assay buffer on ice, centrifuged at 1000g 4 °C for 30 minutes, take the supernatant into a new centrifuge tube. Centrifuged at 10000g 4 °C for 30 minutes, discard the supernatant. Add 1 ml assay buffer into the precipitate on ice. Mix and shock, keep it on ice for detection.

2. For serum, plasma and other biological fluids samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Diluent	--	--	10 µl
Substrate	150 µl	150 µl	150 µl
Mix, then put it in the oven, 37 °C for 10 minutes.			
Dye Reagent	40 µl	40 µl	40 µl
Mix, then put it in the oven, 37 °C for 5 minutes, record absorbance measured at 490 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Monoamine Oxidase activity is defined as the enzyme produces 1 μmol H_2O_2 per minute.

1. According to the protein concentration of sample

$$MAO\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$MAO\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

$$MAO\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 10 mmol/L = 10 μmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$V_{Standard}$: the volume of standard, 0.01 ml;

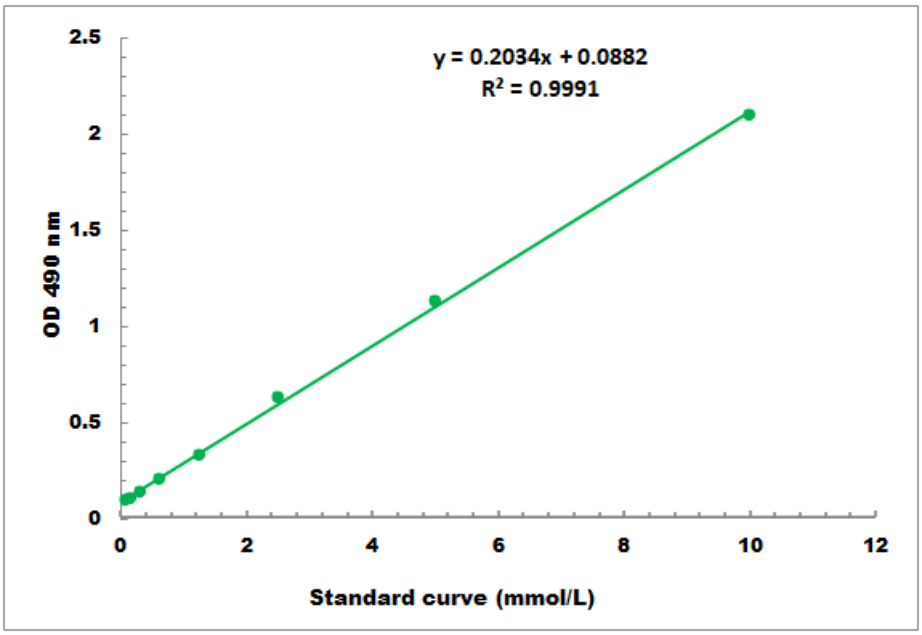
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 5 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES