Monoamine Oxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1102

(Version 1.3D)

Detection and Quantification ofMonoamine Oxidase(MAO) Activityin Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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.

Monoamine Oxidase ActivityColorimetric Microplate Assay Kitprovides a simple and direct procedure for measuring monoamine oxidase activity in a variety of samples.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-WellMicroplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Diluent	20 mlx 1	4 °C
Substrate	Powderx 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	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Substrate: Add 15 ml Diluentto dissolve before use.Keep in dark and store at 4 °C for 1-2 weeks or -20°C for 4-5months after reconstitution.

Dye Reagent: Add 4 ml Diluentto dissolve before use.Keep in dark for immediate use or store at -20°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Bufferon 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 mlAssay 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.

VI. 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit ofMonoamine Oxidaseactivity is defined as the enzymeproduces 1μmolH_2O_2 per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample}-OD_{Blank}) - \text{Intercept}}{\text{Slope}\times T} \times n(U/mL)$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

$$\text{Activity}= \frac{(C_{Standard}\times V_{Standard}) \times (OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank}) \times V_{Sample}\times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

$C_{Standard}$: the concentration of standard, mmol/L= μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

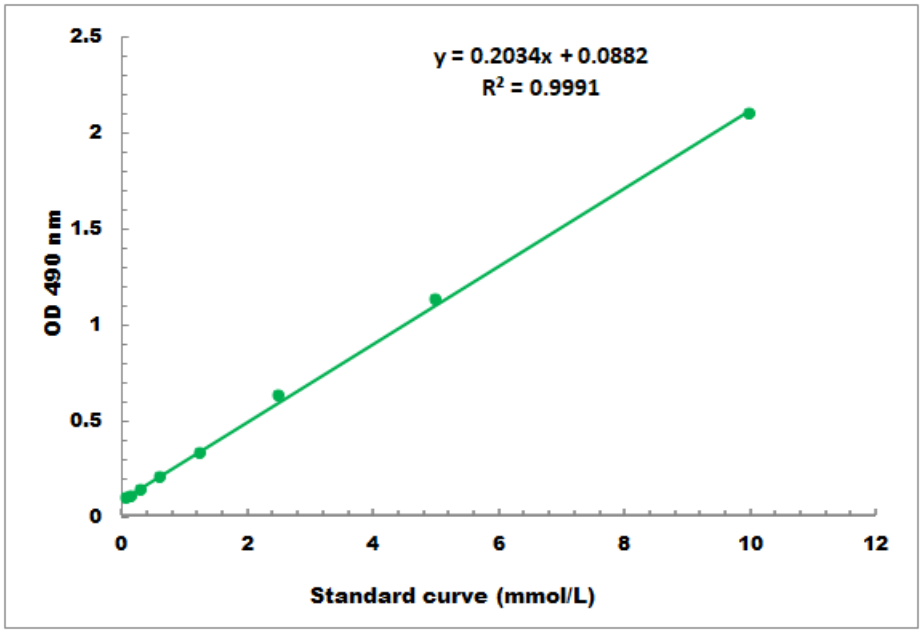
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

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



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