D-Amino Acid Oxidase

Microplate Assay Kit

User Manual

Catalog # CAK1281

(Version 1.1A)

Detection and Quantification of D-Amino Acid Oxidase(DAAO) 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

D-Amino acid oxidase catalyzes the oxidation of D-amino acids as shown below:

$$RCHNH_2COOH + O_2 + H_2O \xrightarrow{\text{D-AAO}} RCOCOOH + NH_3 + H_2O_2$$

The D isomers of alanine, methionine, valine, isoleucine, phenylalanine and proline serve as good substrates while the L isomers do not react at all. The enzyme is a flavoprotein. D-Amino acid oxidase has several possible applications such as the determination of D-amino acids, the separation of natural L- amino acid isomers from a racemic mixture and in the preparation of keto acids.

D-Amino Acid Oxidase Microplate Assay Kit provides a simple and direct procedure for measuring D-Amino Acid Oxidase activity in a variety of samples.In this colorimetric D-Amino Acid Oxidasequantification assay, D-Amino Acid Oxidaseoxidates NADH to NAD+, which then interacts with a specific probe to produce a color. The rate of decrease in the absorbency at 450 nm, is a measure of D-Amino Acid Oxidase activity.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Enzyme	Powder x 1	-20 °C
Dye ReagentA	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
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Note:

Dye Reagent A: add 9 mldistilled water to dissolve before use, mix; store at -20 °C for a monthafter reconstitution.

Substrate: add 1 mlReaction Buffer to dissolve before use; store at -20 °C for a weekafter reconstitution.

Enzyme: add 1 mlReaction Buffer to dissolve before use; store at -80 °C for a monthafter reconstitution.

Standard:add 1 mldistilled water to dissolve before use; then add 0.15 ml into 0.85 mldistilled water, the concentration will be 300 µmol/L; store at -20 °C for a weekafter reconstitution.

Positive Control: add 1 ml Assay Bufferto dissolve before use store at -80 °C for a monthafter reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay bufferfor 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Sample	10 µl	--	--	--	
Standard	--	--	100 µl	--	
Positive Control					10 µl
Distilled water	--	10 µl	--	100 µl	
Reaction Buffer	70 µl	70 µl	--	--	70 µl
Substrate	10 µl	10 µl	--	--	10 µl
Enzyme	10 µl	10 µl			10 µl
Mix.					
Dye Reagent A	90 µl	90 µl	90 µl	90 µl	90 µl
Dye ReagentB	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, incubate at room temperature for 5 minutes,recordabsorbance measured at 450 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 DAAOactivity is defined asthe enzyme oxidates 1nmolNADH per minute.

1. According to the volume of serum or plasma

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

$$= 600 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$$

2.According to the protein concentration of sample

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

$$= 600 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

3.According to the weight of sample

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

$$= 600 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / W$$

4.According to the quantity of cell or bacteria

$$DAAO\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 600 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the concentration of standard, 300 μmol/L = 300nmol/ml;

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

W: the weight of sample, g;

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

$V_{Standard}$: the volume of standard, 0.1 ml;

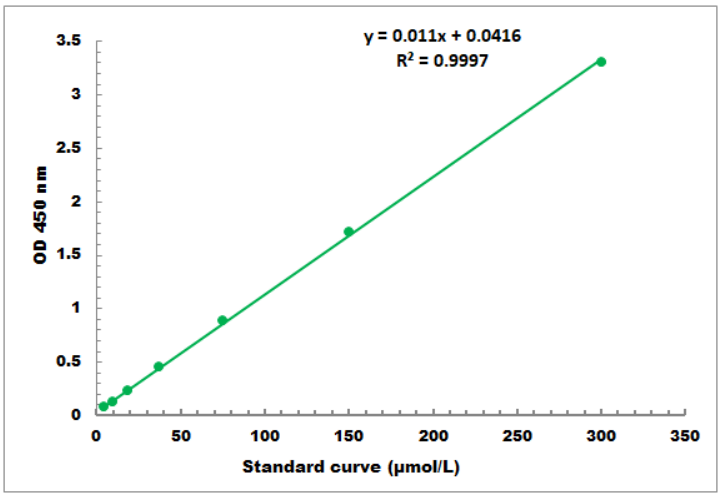
V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 5 minutes;

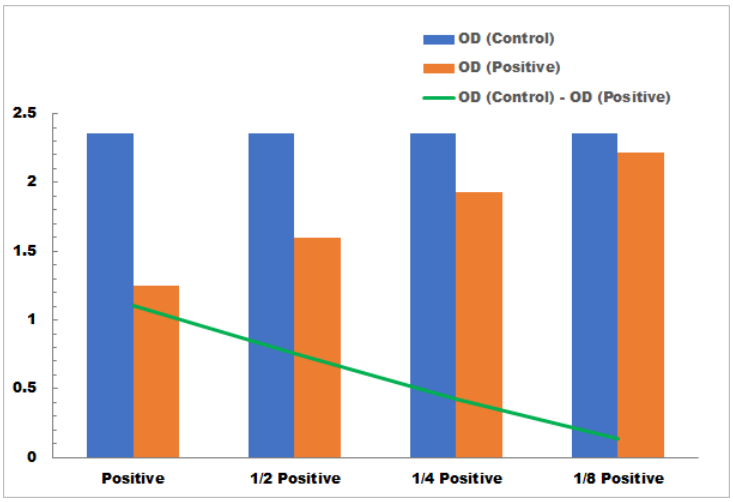
N: the quantity of cell or bacteria, N $\times 10^4$.

VII. TYPICAL DATA

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



Detection Range: 3μmol/L -300 μmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

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

IX. NOTES