Diamine Oxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1055

(Version 1.2F)

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

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

I. INTRODUCTION

Diamine oxidase (DAO) is an enzyme that your body uses to break down ingested histamine. There are a wide variety of foods that contain histamine, and it is DAO's job to break this histamine down. DAO also helps with the integrity of the gut lining, protecting us from leaky gut and the functional digestive issues that can precipitate from it.

The assay is initiated with the enzymatic catalysis of cadaverine by DAO. The enzyme catalysed reaction products dianisidine can be measured at a colorimetric readout at 460 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Substrate	Powder x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	1 ml x 1	4 °C
Standard (5 mmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Assay Buffer to dissolve before use.

Substrate: add 1 ml distilled water to dissolve before use.

Dye Reagent: add 1 ml Dye Reagent Diluent to dissolve before use.

Positive Control: add 1 ml distilled water to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 460 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 cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 12000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 12000g 4 °C for 20 minutes, 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 in the microplate:

Reagent	Sample	Standard	Blank	Positive Control
Sample	20 µl	--	--	20 µl
Distilled water	--	--	20 µl	--
Reaction Buffer	150 µl	150 µl	150 µl	150 µl
Enzyme	10 µl	10 µl	10 µl	10 µl
Substrate	10 µl	10 µl	10 µl	10 µl
Standard	--	20 µl	--	--
Dye Reagent	10 µl	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 30 minutes, measured at 460 nm and record the absorbance.				

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 DAO is defined as the enzyme generates 1 μmol H2O2 per minute at pH7.2, 37 °C.

1. According to the protein concentration of sample

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

$$= 0.167 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.167 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= 0.167 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

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

$$= 0.167 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the concentration of Standard, 5 mmol/L = 5 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$V_{Standard}$: the volume of standard, 0.02 ml;

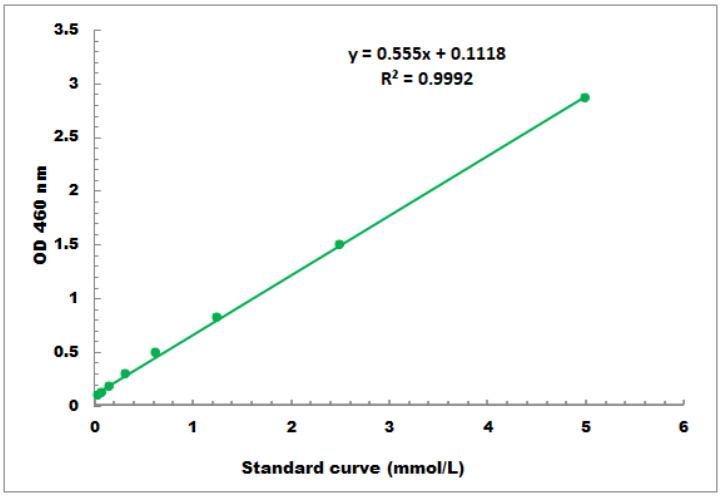
V_{Sample}: the volume of sample, 0.02 ml;

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

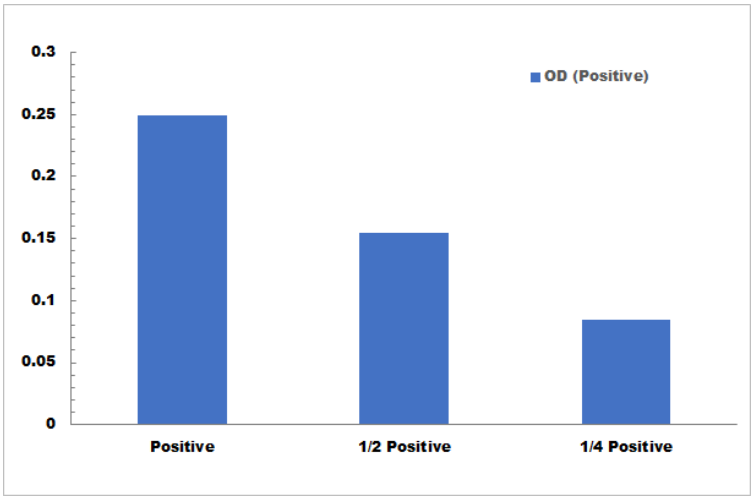
T: the reaction time, 30 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.05 mmol/L - 5 mmol/L



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

VIII. TECHNICAL SUPPORT

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

IX. NOTES