Diamine Oxidase Activity Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6662

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 .

1. 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.

Diamine Oxidase Activity Microplate Assay Kit provides a simple and direct procedure for measuring diamine oxidase activity in a variety of samples. 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.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 mL x 1	4 °C
Enzyme	Powderx 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	-

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening.Perform 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 mL Assay Bufferto dissolve before use.Store at -80°C for 1 month after reconstitution.

Substrate: Briefly centrifuge prior to opening. Add 1 mL distilled water to dissolve before use.Store at 4°C for 1-2 weeks or -20°C for 2 -3months after reconstitution.

Dye Reagent: Briefly centrifuge prior to opening. Add 1 mL Dye Reagent Diluentto dissolve before use.Keep in dark and store at 4°C for 1-2 months or -20°C for 6-7months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 mL distilled water to dissolve before use.Store at -80°C for 1 month after reconstitution.

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

5. 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 12000 g 4°C for 20minutes , 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 12000 g 4°C for 2 0minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples Detect directly.

6. 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 °Cfor 30 minutes,measured at 460 nm and recordthe absorbance.				

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.

7. Calculation

Unit Definition:

One unit of DAO is defined as the enzyme generates 1μmol H_2O_2 per minute at pH7.2, 37 °C .

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

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

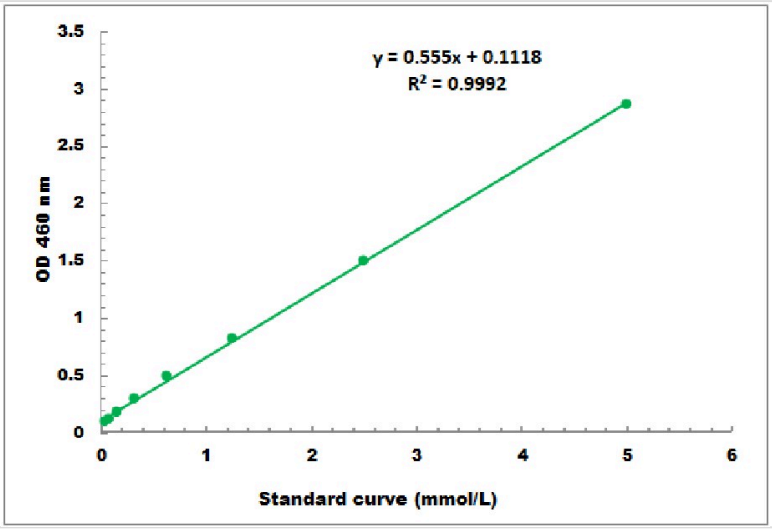
2.4 According to the volume of sample

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

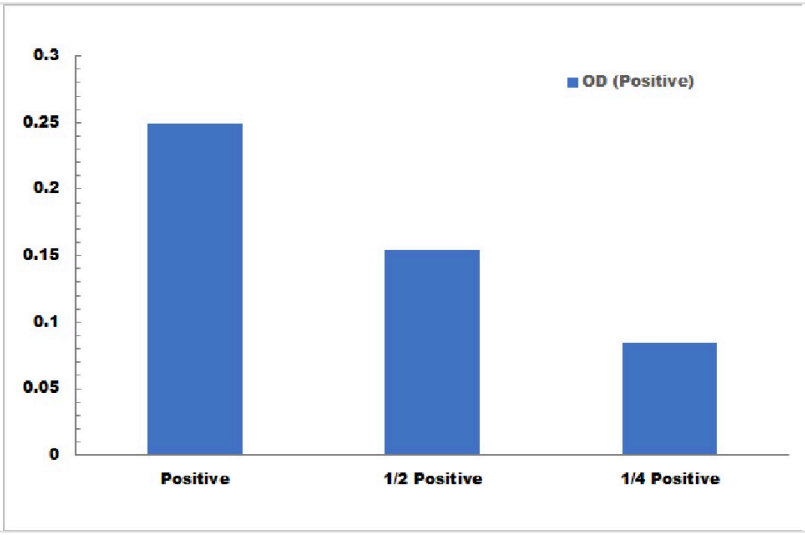
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, mmol/L = μmol/mL
$V_{Standard}$	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

8. Typical Data

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



Dete ction Range : 0.05 mmol/L - 5 mmol/L



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