Diamine Oxidase Microplate Assay Kit

Catalog # AS0055

Detection and Quantification of Dehydroascorbate Reductase Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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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	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
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Assay Bufferto dissolve before use.

Substrate: add 1 ml distilled waterto dissolve before use.

Dye Reagent: add 1 ml Dye Reagent Diluentto dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 460 nm
2. Distilled water
3. 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 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 12000g 4°C for 20minutes, 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 12000g 4°C for 20minutes, 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
Sample	20 µl	--	--
Distilled water	--	--	20 µl
Reaction Buffer	150 µl	150 µl	150 µl
Enzyme	10 µl	10 µl	10 µl
Substrate	10 µl	10 µl	10 µl
Standard	--	20 µl	--
Dye Reagent	10 µl	10 µl	10 µl
Mix, put it in the oven,37 °Cfor 30 minutes,measured at 460 nm and recordthe absorbance.			

VI. CALCULATION

Unit Definition: One unit of DAO is the enzyme that generates 1μmolH2O2 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 = 5μmol/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;

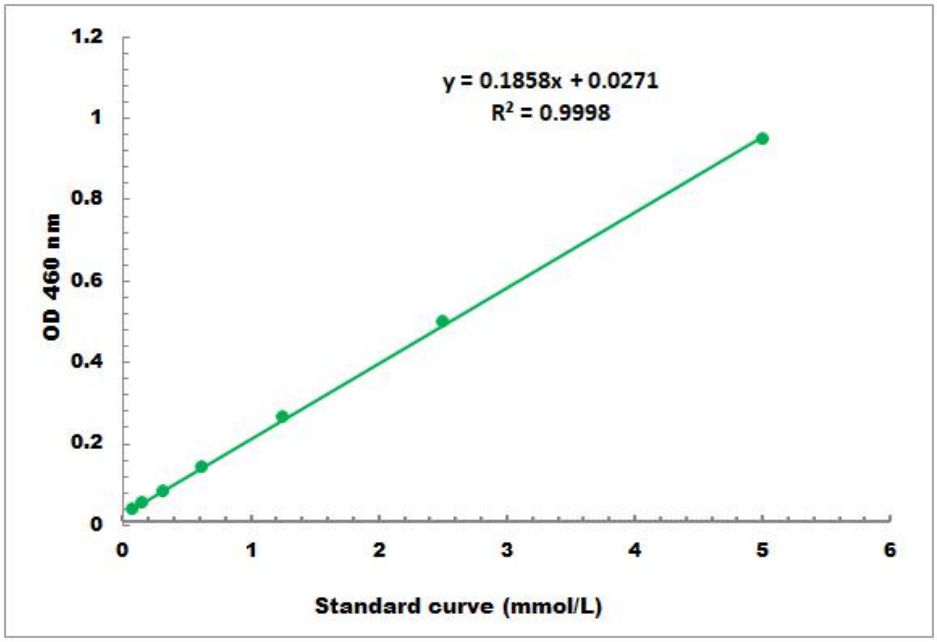
V_{Sample}: the volume of sample, 0.02 ml;

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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES