



Diamine Oxidase Microplate Assay Kit

User Manual

Catalog # ASK1055

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.

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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	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
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.

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 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 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
Sample	20 μ l	--	--
Distilled water	--	--	20 μ l
Reaction Buffer	150 μ l	150 μ l	150 μ l
Enzyme	10 μ l	10 μ l	10 μ l
Substrate	10 μ l	10 μ l	10 μ l
Mix, put it in the oven, 37 °C for 30 minutes.			
Standard	--	20 μ l	--
Dye Reagent	10 μ l	10 μ l	10 μ l
Mix, wait for 2 minutes, measured at 460 nm and record the absorbance.			

VI. CALCULATION

Unit Definition: One unit of DAO is the enzyme that generates 1 μmol H₂O₂ per minute at pH7.2, 37 °C.

1. According to the protein concentration of sample

$$\begin{aligned}\text{DAO (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times C_{\text{Protein}}) / T \\ &= 0.167 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}}\end{aligned}$$

2. According to the weight of sample

$$\begin{aligned}\text{DAO (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 0.167 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W\end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned}\text{DAO (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (N \times \\ &\quad V_{\text{Sample}} / V_{\text{Assay}}) / T \\ &= 0.167 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N\end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned}\text{DAO (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 0.167 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})\end{aligned}$$

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

C_{Standard} : the concentration of Standard, 5 mmol/L = 5 $\mu\text{mol/ml}$;

W: the weight of sample, g;

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

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

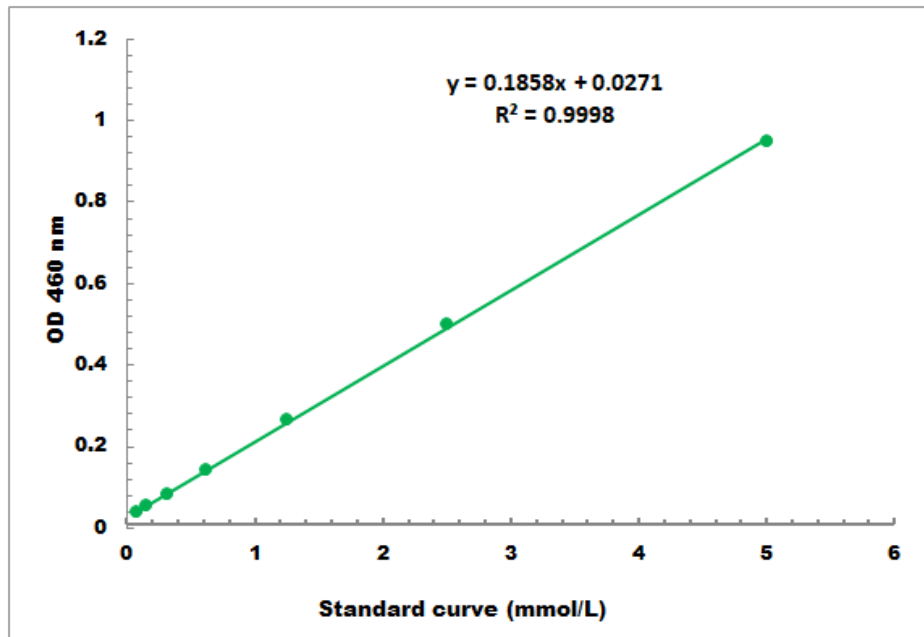
V_{Sample} : the volume of sample, 0.02 ml;

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