



Diamine Oxidase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1055

(Version 1.3F)

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

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.

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.

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
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

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

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to 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 2-3 months after reconstitution.

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

V. 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.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

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

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of DAO is defined as the enzyme generates 1 $\mu\text{mol H}_2\text{O}_2$ per minute at pH 7.2, 37 °C.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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 = $\mu\text{mol/mL}$

V_{Standard} : the volume of standard in assay procedure, mL

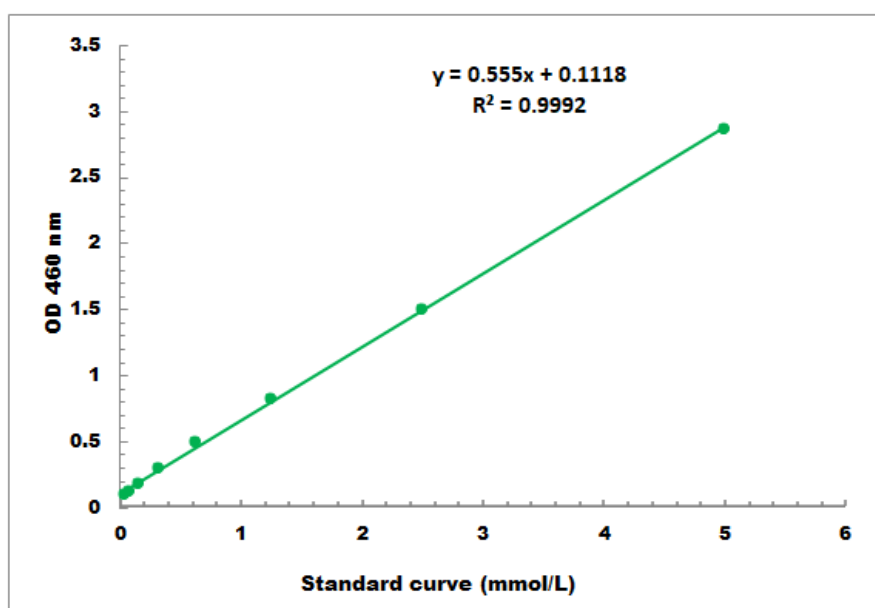
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

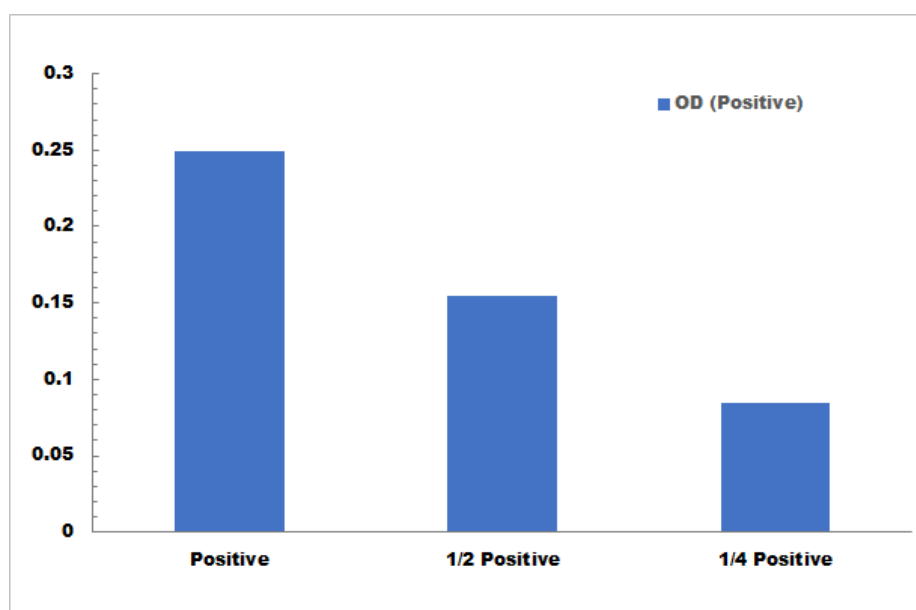
T: the reaction time, minute

VIII. 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