Diamine Oxidase Assay Kit

Diamine Oxidase Assay Kit is a detection kit for the quantification of Diamine Oxidase Activity in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82024

Package: 96 wells

TABLE OF CONTENTS

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INTRODUCTION

Diamine oxidase (DAO), also known as histaminase, is an enzyme (EC 1.4.3.22) involved in the metabolism, oxidation, and inactivation of histamine and other polyamines such as putrescine or spermidine in animals.

The highest levels of DAO expression are observed in the digestive tract and the placenta. In humans, a certain subtype of cells of the placenta, namely the extravillous trophoblasts, express the enzyme and secrete it into the blood stream of a pregnant woman. Lowered diamine oxidase values in maternal blood in early pregnancy might be an indication for trophoblast-related pregnancy disorders like early-onset preeclampsia. Normally the enzyme is not or only very scarce present in the blood circulation of humans, but it increases vastly in pregnant women suggesting a protective mechanism against adverse histamine. It is also secreted by eosinophils. In case of a shortage of diamine oxidase or low DAO activity in the human body, it may appear as an allergy or histamine intolerance. [Wikipedia Diamine oxidase]

PRINCIPLE OF THE ASSAY

This kit is designed to measure Diamine Oxidase (DAO) Activity in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants. H2O2 is produced from Diamine Oxidase reaction. And the H2O2 is then used by HRP to oxidize a dye making it to be read out at 460 nm. The Diamine Oxidase (DAO) Activity in the sample is then determined by comparing the O.D. of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, Enzyme should be store at-20°C. Store all other components at 4°C at all time.

Component	Quantity	Storage information
Microplate	96 wells	4°C
Standard (5 mmol/L)	1 ml	4°C
Positive control	1 vial (Lyophilized)	-20°C
Enzyme	1 vial (Lyophilized)	-20°C
Assay Buffer	4 X 30 ml (Ready to use)	4°C
Reaction Buffer	20 ml (Ready to use)	4°C
Substrate	1 vial (Lyophilized)	4°C
Reaction Dye	1 vial (Lyophilized)	4°C
Reaction Dye Diluent	1 ml (Ready to use)	4°C
Plate sealer	3 strips	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 460 nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, Enzyme should be store at -20°C. Store all other components at 4°C at all time.
- Reconstituted Enzyme should be aliquoted and stored at-20°C to avoid repeated freeze-thaw cycles.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at ≤-20°C or below. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20°C or below. Avoid repeated freeze-thaw cycles.

Tissue lysate- Weigh out 0.1 g of tissue, then tissues are homogenized with 1 ml Assay buffer on ice. Centrifuge homogenized tissues at 12,000 g for 20 minutes at 4°C. Collect the supernatant into a new centrifuge tube and keep it on ice for detection. Assay immediately or aliquot and store samples at ≤-20°C or below. Avoid repeated freeze-thaw cycles.

Cell lysate- Rinse the cells with ice-cold PBS. Scrape the cells or trypsinize cells and the wash once by ice-cold PBS. Centrifuge the samples at ~300-3000 x g for 2-5 minutes to remove PBS. Add 1 ml Assay buffer pre 5 X 10^6 cells to resuspend the cells. Sonicate the samples with power set at 20% for 3 sec and pauses for 10sec, repeat for 30 times. Be careful the sample should keep on ice while sonication. Centrifuge samples at 12,000 g for 20 minutes at 4°C. Collect the supernatant into a new centrifuge tube and keep it on ice for detection. Assay immediately or aliquot and store samples at ≤-20°C or below. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants, urine- Remove particulates by centrifugation and aliquot & store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Note:

Samples should be clear and free of precipitates or turbidity. If not, centrifuge or filter to clarify samples prior to assay.

REAGENT PREPARATION

- **Substrate:** Reconstitute the Substrate with **1 ml** of **distilled water**. Allow the Substrate to sit in the tube for few minutes with gentle agitation to make sure the Substrate is dissolved completely before use. Aliquot & store the reconstituted Substrate at 4°C. Avoid repeated freeze-thaw cycles.
- **Reaction Dye:** Reconstitute the Reaction Dye with **1 ml** of **Reaction Dye Diluent**. Allow the Reaction Dye to sit for few minutes with gentle agitation to make sure the Reaction Dye is dissolved completely before use. Aliquot & store the reconstituted Reaction Dye at 4°C. Avoid repeated freeze-thaw cycles.
- **Enzyme:** Reconstitute the Enzyme with **1 ml** of **Assay Buffer.** Allow the Enzyme to sit for few minutes on ice with gentle agitation to make sure the Enzyme is dissolved completely before use. Aliquot & store the reconstituted Enzyme at-20°C. Avoid repeated freeze-thaw cycles.
- **Positive Control:** Reconstitute the Positive Control with **1 ml** of **distilled water**. Allow the Positive Control to sit in the tube for few minutes with gentle agitation to make sure the Positive Control is dissolved completely before use. Aliquot & store the reconstituted Positive Control at-20°C. Avoid repeated freeze-thaw cycles.
- **Sample**: If the initial assay found samples contain Diamine Oxidase activity higher than the standard, the samples can be diluted with **Assay Buffer** and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

(It is recommended to do pre-test to determine the suitable dilution factor).

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **20 µl** per well of **samples** and **Positive Control** into appropriate wells of the plate.
2. Add **20 µl** per well of **samples** and **20 µl** of **distilled water** in sample control wells as sample background control.
3. Add **20 µl** per well of **standard** into appropriate wells of the plate.
4. Add **20 µl** of **distilled water** in Blank well.
5. Add **150 µl** of **Reaction Buffer** per well into all reaction wells.
6. Add **10 µl** of **Substrate** and **10 µl** of **Enzyme** per well into sample, Standard and blank wells.
7. Add **10 µl** per well of **reconstituted Reaction Dye** into all reaction wells.
8. Gently tap plate or shake briefly to mix thoroughly. Incubate the plate for **30 min at 37°C**.
9. Read O.D. with a microplate reader at **460 nm** immediately.

Summary of Assay Procedure

Reagent	Sample	Sample Control	Standard	Blank	Positive Control
Sample	20 µl	20 µl	-	-	-
Positive Control	-	-	-	-	20 µl
Distilled water	-	20 µl	-	20 µl	-
Standard	-	-	20 µl	-	-
Reaction Buffer	150 µl	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
Reaction Dye	10 µl	10 µl	10 µl	10 µl	10 µl
Gently tap plate to mix thoroughly. Incubate the plate for 30 min at 37°C.					
Read O.D. with a microplate reader at 460 nm immediately.					

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

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

3. Calculation:

A. Definition:

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

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

W: the weight of sample, g.

N: the quantity of cell or bacteria, $N \times 10^{4.}$

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

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

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

T: the reaction time, 30 minutes.

B. Formula:

a). According to the protein concentration of sample

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

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

b). According to the weight of sample

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

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

c). According to the quantity of cells or bacteria

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

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

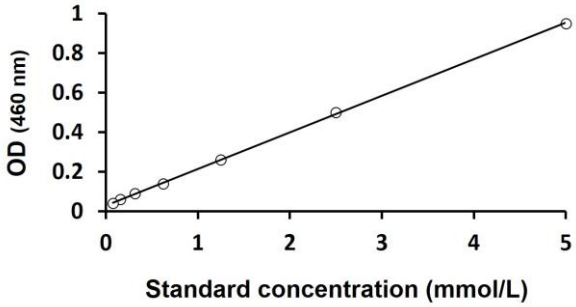
d). According to the volume of serum, plasma

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

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

EXAMPLE OF TYPICAL DATA

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

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