Diamine Oxidase Assay Kit

Catalog No.: abx298821

Size: 100 Assays

Storage: Store the Enzyme and Positive Control at -20°C and all other components at 4°C.

Application: For quantitative detection of Diamine Oxidase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

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

Introduction: Diamine Oxidase, a digestive enzyme synthesized in the kidneys, thymus and intestinal lining of the digestive tract, plays a key role in the oxidative deamination of exogenous, or excess, histidine. In addition, Diamine Oxidase mediates protection against functional digestive issues, via maintaining the structural integrity of the intestinal lining.

Diamine Oxidase catalyzes the oxidative deamination of cadaverine. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 460 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 20 ml
4. Enzyme: 1 vial
5. Dye Reagent: 1 vial
6. Dye Reagent Diluent: 1 ml
7. Standard (5 mmol/L): 1 ml
8. Substrate: 1 vial
9. Positive Control: 1 vial
10. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (460 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Mortar
6. Centrifuge and centrifuge tubes
7. Timer
8. Ice
9. Sonicator

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Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 1 ml of distilled water into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Dye Reagent Solution**
  Add 1 ml of Dye Reagent Diluent into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

- **Enzyme Solution**
  Add 1 ml of Assay Buffer to the Standard vial and mix thoroughly to prepare the Enzyme solution. Ensure that the Enzyme has completely dissolved prior to use.

- **Positive Control Solution**
  Add 1 ml of distilled water to the Positive Control vial and mix thoroughly to prepare the Positive Control Solution. Ensure that the Positive Control has completely dissolved prior to use.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 12,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice, then allow to stand for 2 hours. Centrifuge at 12,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

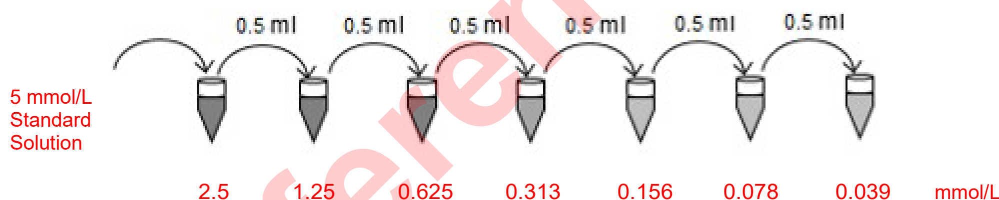
- **Serum and Plasma samples**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 7 tubes with 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.313 mmol/L, 0.156 mmol/L, 0.078 mmol/L and 0.039 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 5 mmol/L standard solution to the 1st tube (2.5 mmol/L). Add 0.5 ml of the 2.5 mmol/L standard solution to the 2nd tube (1.25 mmol/L) and mix thoroughly. Transfer 0.5 ml from the 2nd tube to the 3re tube and mix thoroughly, and so on.



2. Set the sample, standard, positive control and blank wells on the microplate. We recommend setting up each standard and sample in duplicate.
3. Add 20 µl of sample to the sample wells and positive control wells.
4. Add 20 µl of distilled water to the blank wells.
5. Add 150 µl of Reaction Buffer to all wells.
6. Add 10 µl of Enzyme Solution to all wells.
7. Add 10 µl of Substrate Solution to all wells.
8. Add 20 µl of prepared standards to the standard wells.
9. Add 10 µl of Dye Reagent Solution to all wells.
10. Tap the plate gently to mix and incubate at 37°C for 30 minutes.
11. Read and record absorbance at 460 nm.

C. Calculations

One unit of Diamine Oxidase activity is defined as the amount of enzyme required to produce 1 µmol of H_2O_2 per minute.

Diamine Oxidase activity per mg of protein:

$$\text{Diamine Oxidase (U/mg)} = \frac{C_{Standard} \times V_{Standard}}{V_{Sample} \times C_{Protein} \times T} \times \frac{OD_{Sample} - OD_{blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.167}{C_{Protein}} \times \frac{OD_{Sample} - OD_{blank}}{OD_{Standard} - OD_{Blank}}$$

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Diamine Oxidase activity per g of sample:

$$\textbf{Diamine Oxidase (U/g)} = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times W \times T} \times \frac{OD_{Sample} - OD_{blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.167}{W} \times \frac{OD_{Sample} - OD_{blank}}{OD_{Standard} - OD_{Blank}}$$

Diamine Oxidase activity per 10^4 cells or bacteria:

$$\textbf{Diamine Oxidase (U/}10^4\textbf{ cells)} = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times N \times T} \times \frac{OD_{Sample} - OD_{blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.167}{N} \times \frac{OD_{Sample} - OD_{blank}}{OD_{Standard} - OD_{Blank}}$$

Diamine Oxidase activity per ml of sample:

$$\textbf{Diamine Oxidase (U/ml)} = \frac{C_{Standard} \times V_{Standard}}{V_{Sample} \times T} \times \frac{OD_{Sample} - OD_{blank}}{OD_{Standard} - OD_{Blank}} = 0.167 \times \frac{OD_{Sample} - OD_{blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Protein}$ Concentration of protein (in mg/ml)

$C_{Standard}$ Concentration of highest standard (5 mmol/L)

T Reaction time (30 minutes)

W Weight of the sample (in g)

N Number of cells or bacteria (× 10^4)

V_{Assay} Volume of assay buffer used in sample preparation (1 ml)

V_{Sample} Volume of sample (0.02 ml)

$V_{Standard}$ Volume of standard (0.02 ml)

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