



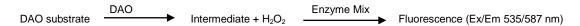
Diamine Oxidase Activity Assay Kit (Fluorometric)

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(Catalog # K496-100; 100 assays; Store at -20°C)

Introduction:

I. Diamine Oxidase (DAO, E.C. 1.4.3.22) is an important regulator of polyamine levels in human physiology. DAO metabolizes the oxidative conversion of biogenic polyamines such as putrescine and cadaverine into aldehydes. Biogenic amines are known regulators of important biological pathways such as cell growth and signal transduction, placing DAO in an important regulatory position in maintaining cell viability. Putrescine itself is also an activator of c-jun and c-fos expression, further linking this diamine with potential roles in cancer development. DAO is notably involved in regulating growth and differentiation of intestinal mucosa, bone marrow, and other rapidly proliferating tissue. In addition to its role in tissue growth, DAO activity is important in metabolism. Loss of diamine oxidase activity can lead to histamine intolerance and histamine poisoning. BioVision's Diamine Oxidase Activity Assay Kit provides a straightforward method to determine DAO activity of tissue lysates as well as recombinant enzymes, with a detection limit of less than 1 pmole/min of activity. In the assay, DAO converts the provided substrate, yielding an intermediate and H₂O₂. H₂O₂ is then utilized by the DAO Enzyme Mix to generate fluorescence (Ex/Em = 535/587 nm) from the DAO Probe.



II. Applications:

- · Measurement of DAO activity in various tissues/cell extracts
- · Characterization of DAO activity of purified recombinant enzyme

III. Sample Type:

- · Animal tissues: intestine, e.g.
- · Purified Enzyme Preparations

IV. Kit Contents:

Components	K496-100	Cap Code	Part Number
DAO Assay Buffer	25 ml	WM	K496-100-1
DAO Probe (in DMSO)	200 µl	Red	K496-100-2
DAO Substrate (Lyophilized)	1 vial	Blue	K496-100-3
DAO Enzyme Mix (Lyophilized)	1 vial	Green	K496-100-4
H ₂ O ₂ Standard (0.88 M)	100 µl	Yellow	K496-100-5
DAO Positive Control (Lyophilized)	1 vial	Orange	K496-100-6

V. User Supplied Reagents and Equipment:

- · Black 96-well plate with flat bottom,
- · Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- DAO Assay Buffer and DAO Probe: Warm to room temperature before use. Store at -20°C.
- DAO Substrate: Reconstitute in 1.1 ml DAO Assay Buffer. Aliquot and store at -20°C. Use within two months.
- DAO Enzyme Mix: Reconstitute with 220 µl DAO Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months.
- H_2O_2 Standard (0.88 M): Ready to use. Store at -20°C. Use within two months.
- DAO Positive Control: Add 44 μl DAO Assay Buffer to the Positive Control and mix thoroughly. Aliquot and store at –20°C. Keep on ice while in use. Use within two months.

VII. Diamine Oxidase Activity Assay Protocol:

1. Sample Preparation: Add 50 μl of DAO Assay Assay Buffer per 10 mg of sample (wet weight or cell pellet). See note (a) below. Homogenize on ice using a Dounce homogenizer (Cat.# 1998). Centrifuge at 10,000 X g for 5 min. at 4°C. Collect the supernatant. Add 2-50 μl of supernatant (to avoid interference, it is recommended to use no more than 10 μg protein/well) to desired wells of black 96-well plate and adjust the volume to 50 μl with DAO Assay Buffer. For each reaction, prepare identical background control reactions in separate wells. For positive control well, add 2 μl of DAO Positive Control and adjust the final volume to 50 μl with DAO Assay Buffer.

Notes

- a. It is recommended to include Protease Inhibitor Cocktail (Cat.# K271-500) when preparing samples from tissue or cell lysate.
- b. Cell & tissue lysate samples can be stored at -80°C for future experiments.
- c. For unknown samples, we recommend doing a pilot experiment testing several doses to ensure that readings are within the range of the standard curve.
- c. For samples exhibiting significant background (i.e. most lysates), prepare parallel sample reactions without the substrate as background controls.
- d. We recommend filtration of small molecules that may interfere with the assay. This can be accomplished by concentrating with BioVision 10k spin column (Cat.# 1997). Spin a desired volume to concentrate the lysate, then dilute it back to the original volume with fresh DAO Assay Buffer.

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- 2. Standard Curve Preparation: Dilute 10 μl H₂O₂ Standard (0.88 M) into 870 μl dH₂O to generate a 10 mM H₂O₂ standard. Further dilute 10 μl of 10 mM H₂O₂ Standard by adding 990 μl dH₂O to generate 0.1 mM H₂O₂ Standard. Add 0, 2, 4, 6, 8 and 10 μl of the diluted H₂O₂ Standard into a series of wells in a black 96-well plate to generate 0, 0.2, 0.4, 0.6, 0.8, and 1.0 nmole per well of H₂O₂ Standard. Bring the total volume in each well to 50 μl with Assay Buffer.
- 3. Reaction Mix: Mix enough reagents for the number of assays to be performed, including standards. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background/Standard Mix
DAO Assay Buffer	36 µl	46 µl
DAO Substrate	10 µl	-
DAO Enzyme Mix	2 µl	2 µl
DAO Probe	2 µl	2 μΙ

Mix and add 50 μ I of the Reaction Mix to each well containing samples and Positive Control. For H_2O_2 , Control and Sample Background wells, mix and add 50 μ I of the Background/Standard Mix. Mix well.

4. Measurement: Incubate plate for 60 min. at 37°C and read fluorescence (Ex/Em = 535/587 nm) in kinetic mode.

Note: For samples containing low DAO activity, longer incubation times may be required.

5. Calculation: Subtract 0 Standard reading from all readings. Plot the H₂O₂ Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate the diamine oxidase activity of the test sample: ΔRFU = RFU_{final} – RFU_{initial}. Apply the ΔRFU to the H₂O₂ Standard Curve to get B nmol of H₂O₂ generated during the reaction time (Δt = t₂ - t₁).

Sample Diamine Oxidase Activity = $B/(\Delta t \times V) \times D = nmol/min/ml = mU/ml$

Where: $\mathbf{B} = H_2O_2$ amount from Standard Curve (nmol).

 $\Delta RFU = RFU_{final} - RFU_{initial}$

 $\Delta \mathbf{t}$ = reaction time (min.)

V = sample volume added into the reaction well (ml)

D = Dilution Factor

Unit Definition: One unit of diamine oxidase (DAO) is the amount of enzyme that generates 1.0 μ mol of H₂O₂ per min. at pH 7.4 at 37°C.

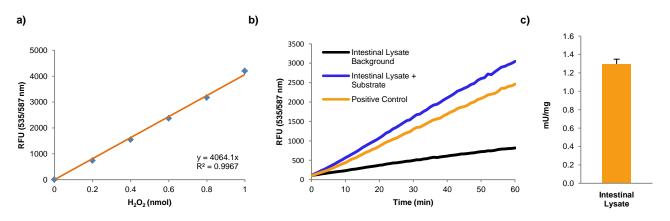


Figure: a) H_2O_2 standard curve; (b) Kinetic measurement of DAO activity from Positive Control (c) Activity determination of intestinal tissue lysate. For this experiment, 100 mg rat intestine was used, following Diamine Oxidase Activity Assay Kit protocol and including protease inhibitor in the lysis. Lysate (7 μ g) was assayed and specific activity was determined to be 1.30 nmol/min/mg lysate.

VIII. RELATED PRRFUUCTS:

Monoamine Oxidase (Total MAO/MAO-A/MAO-B) Fluorometric Assay Kit (K795) Monoamine Oxidase A (MAO-A) Inhibitor Screening Kit (Fluorometric) (K796) Monoamine Oxidase B (MAO-B) Inhibitor Screening Kit (Fluorometric) (K797) Clorgyline hydrochloride (2622) Moclobemide (9561) Pargyline hydrochloride (2618)

FOR RESEARCH USE ONLY! Not to be used on humans.