Version 1 Last updated 24 October 2018

ab241004 Diamine Oxidase Assay Kit (Fluorometric)

For the detection of Diamine Oxidase activity in purified enzyme preparations and tissues/cell extracts.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Diamine Oxidase Assay Kit (ab241004) provides a straightforward method to determine DAO activity of tissue lysates as well as recombinant enzymes, with a detection limit of less than 1 pmol/min of activity.

In the assay, DAO converts the provided substrate, yielding an intermediate and H_2O_2. H_2O_2 is then utilized by the DAO Enzyme Mix to generate fluorescence (Ex/Em = 535/587 nm) from the DAO Probe.

2. Protocol Summary

Prepare tissue or cell samples and positive/background control.

Prepare standard curve.

Prepare reaction mix and add to standards, positive control and sample wells.

Measure fluorescence (Ex/Em = 535/587 nm) immediately in kinetic mode for 1 hour at 37°C.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
DAO Assay Buffer	25 mL	-20°C
DAO Probe (in DMSO)	200 µL	-20°C
DAO Substrate (Lyophilized)	1 vial	-20°C
DAO Enzyme Mix (Lyophilized)	1 vial	-20°C
H_2O_2 Standard (0.88 M)	100 µL	-20°C
DAO Positive Control (Lyophilized)	1 vial	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 DAO Assay Buffer:

Ready to use as supplied. Bring to room temperature before use. Store at -20°C.

6.2 DAO Probe (in DMSO):

Ready to use as supplied. Bring to room temperature before use. Store at -20°C.

6.3 DAO Substrate (Lyophilized):

Reconstitute in 1.1 mL DAO Assay Buffer. Aliquot and store at -20°C. Use within two months.

6.4 DAO Enzyme Mix (Lyophilized):

Reconstitute with 220 µL DAO Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months.

6.5 H_2O_2 Standard (0.88 M):

Ready to use as supplied. Store at -20°C. Use within two months.

6.6 DAO Positive Control (Lyophilized):

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.

7. Standard Preparation

– Always prepare a fresh set of standards for every use.

7.1 Dilute 10 µL H_2O_2 Standard (0.88 M) into 870 µL dH_2O to generate a 10 mM H_2O_2 standard.

7.2 Further dilute 10 µL of 10 mM H_2O_2 Standard by adding 990 µL dH_2O to generate 0.1 mM H_2O_2 Standard.

7.3 Add 0, 2, 4, 6, 8 and 10 µL of the diluted H_2O_2 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 nmol per well of H_2O_2 Standard.

7.4 Bring the total volume in each well to 50 µL with Assay Buffer.

Standard #	H_2O_2 0.1 mM Standard (µL)	DAO Assay Buffer (µL)	H_2O_2 Standard nmol/well
1	0	50	0
2	2	48	0.2
3	4	46	0.4
4	6	44	0.6
5	8	42	0.8
6	10	40	1.0

8. Sample Preparation

8.1 Add 50 µL of DAO Assay Buffer per 10 mg of sample (wet weight or cell pellet). Homogenize on ice using a dounce homogenizer.

8.2 Centrifuge at 10,000 X *g* for 5 min. at 4°C. Collect the supernatant.

8.3 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.

8.4 For each reaction, prepare identical background control reactions in separate wells.

8.5 For positive control well, add 2 µL of DAO Positive Control and adjust the final volume to 50 µL with DAO Assay Buffer.

Δ Note:

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

9. Assay Procedure

DAO Substrate Solution Preparation:

9.1 Mix enough reagents for the number of assays to be performed, including standards.

9.2 For each well, prepare 50 µL Mix containing:

Component	Reaction mix (µL)	Background/ Standard Mix (µL)
DAO Assay Buffer	36	46
DAO Substrate	10	---
DAO Enzyme Mix	2	2
DAO Probe	2	2

9.3 Mix and add 50 µL of the Reaction Mix to each well containing samples and Positive Control.

9.4 For H_2O_2, Control and Sample Background wells, mix and add 50 µL of the Background/Standard Mix.

9.5 Mix well.

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

Δ Note: Incubation time depends on the DAO activity in the samples. Longer incubation time may be required for samples having low DAO activity.

10. Data Analysis

10.1 Subtract 0 Standard reading from all readings.

10.2 Plot the H_2O_2 Standard Curve.

10.3 If sample background control reading is significant, subtract the background control reading from its paired sample reading.

10.4 Calculate the diamine oxidase activity of the test sample:

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

10.5 Apply the ΔRFU to the H_2O_2 Standard Curve to get B nmol of H_2O_2 generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\text{Sample DAO Activity} = \mathbf{B/(\Delta T \times V) \times D} = \text{nmol/min/mL} = \text{mU/mL}$$

Where:

B is H_2O_2 amount in the sample well from Standard Curve (nmol).
ΔT is reaction time (min).
V is sample volume added into the reaction well (mL)
D is sample dilution factor (D=1 when samples are undiluted)

Unit Definition: One unit of diamine oxidase (DAO) is the amount of enzyme that generates 1.0 µmol of H_2O_2 per min. at pH 7.4 at 37°C.

11. Typical Data

Typical data provided **for demonstration purposes only**.

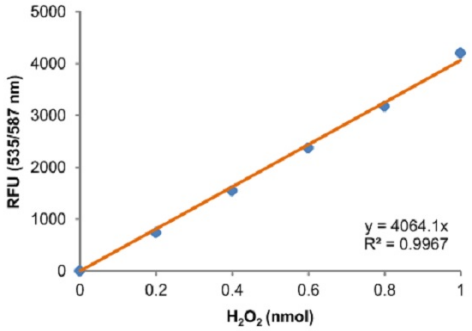

Figure 1. H_2O_2 Standard Curve.

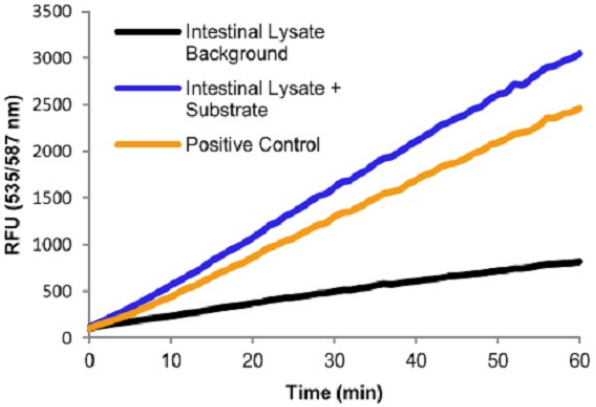

Figure 2. Kinetic measurement of DAO activity from Positive Control.

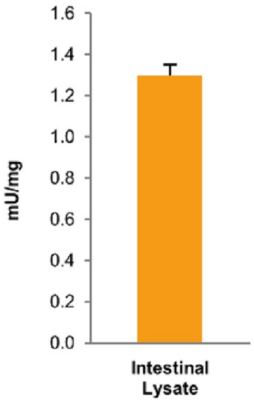

Figure 3. Activity determination of intestinal tissue lysate. For this experiment, 100 mg rat intestine was used, following Diamine Oxidase Activity Assay Kit protocol and including protease inhibitor in the lysis. Lysate (7 µg) was assayed and specific activity was determined to be 1.30 nmol/min/mg lysate.

13. Notes

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