3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Diamine Oxidase Activity Assay Kit

Catalog Number **MAK351**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Diamine Oxidase (DAO) 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.

The 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/minute 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 (λ_{ex} = 535 nm/λ_{em} = 587 nm) from the DAO Probe.

This kit is suitable for the measurement of DAO activity in various tissues and cell extracts as well as the characterization of DAO activity of purified recombinant enzyme.

Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

DAO Assay Buffer Catalog Number MAK351A	25 mL
DAO Probe (in DMSO) Catalog Number MAK351B	200 µL
DAO Substrate (Lyophilized) Catalog Number MAK351C	1 vial
DAO Enzyme Mix (Lyophilized) Catalog Number MAK351D	1 vial
H_2O_2 Standard (0.88 M) Catalog Number MAK351E	100 µL
DAO Positive Control (Lyophilized) Catalog Number MAK351F	1 vial

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black flatbottom 96 well plates
- Fluorescence multiwell plate reader, capable of 37 °C temperature setting
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
- Protease Inhibitor Cocktail (Catalog Number P8340)

Precautions and Disclaimer

For R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions.

DAO Assay Buffer and DAO Probe – Warm to room temperature before use. Store at –20 °C.

DAO Substrate – Reconstitute in 1.1 mL of DAO Assay Buffer. Aliquot and store at –20 °C. Use within two months once reconstituted.

DAO Enzyme Mix – Reconstitute with 220 µL of DAO Assay Buffer. Store at –20 °C. Keep on ice while in use. Use within two months once reconstituted.

DAO Positive Control – Add 44 µL of 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 once reconstituted.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Procedure

Sample Preparation

Notes:

- Cell and tissue lysate samples can be stored at –80 °C for future experiments.
- For unknown samples, it is suggested to perform 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.
- Filtration of small molecules that may interfere with the assay is suggested. This can be accomplished by concentrating with a Corning Spin-X UF concentrator. Spin a desired volume to concentrate the lysate, then dilute it back to the original volume with fresh DAO Assay Buffer.

1. Add 50 µL of DAO Assay Assay Buffer per 10 mg of sample (wet weight or cell pellet). It is suggested to include a Protease Inhibitor Cocktail when preparing samples from tissue or cell lysate.
2. Homogenize on ice using a Dounce homogenizer.
3. Centrifuge at 10,000 × *g* for 5 minutes at 4 °C.
4. Collect the supernatant.
5. 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.
6. For each reaction, prepare identical background control reactions in separate wells.
7. For positive control well, add 2 µL of DAO Positive Control and adjust the final volume to 50 µL with DAO Assay Buffer.

Standard Curve Preparation

1. Prepare a 10 mM H_2O_2 standard by diluting 10 µL of H_2O_2 Standard (0.88 M) into 870 µL of ultrapure water.
2. Further dilute 10 µL of 10 mM H_2O_2 Standard by adding 990 µL of ultrapure water to generate 0.1 mM H_2O_2 Standard.
3. Prepare H_2O_2 Standards in desired wells of black 96 well plate according to Table 1. Mix well.

Table 1.
Preparation of H_2O_2 Standards

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

Reaction Mix

Mix enough reagents for the number of assays to be performed, including standards. For each well, prepare 50 µL according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mixes

Reagent	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 µL

Add 50 µL of the Reaction Mix to each well containing samples and Positive Control. For H_2O_2 Standards, Control, and Sample Background wells, add 50 µL of the Background/Standard Mix. Mix well.

Measurement

Incubate the plate at 37 °C for 60 minutes (for samples containing low DAO activity, longer incubation times may be required). Measure the fluorescence at λ_{ex} = 535 nm/λ_{em} = 587 nm in kinetic mode.

Results

1. Subtract 0 Standard reading from all readings.
2. Plot the H_2O_2 Standard Curve.
3. If sample background control reading is significant, subtract the background control reading from its paired sample reading.
4. Calculate the diamine oxidase activity of the test sample: $\Delta RFU = RFU_{final} - RFU_{initial}$.
5. Apply the ΔRFU to the H_2O_2 Standard Curve to get B nmol of H_2O_2 generated during the reaction time ($\Delta t = t_2 - t_1$).

Sample DOA Activity (nmol/min/µL or mU/µL) =

$$(B/(\Delta t \times V)) \times D$$

where:
B = H_2O_2 amount from Standard Curve (nmol)
ΔRFU = RFU_{final} - $RFU_{initial}$
Δt = reaction time (minutes)
V = sample volume added into the reaction well (µL)
D = Dilution Factor

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

Figure 1.
Typical H_2O_2 Standard Curve

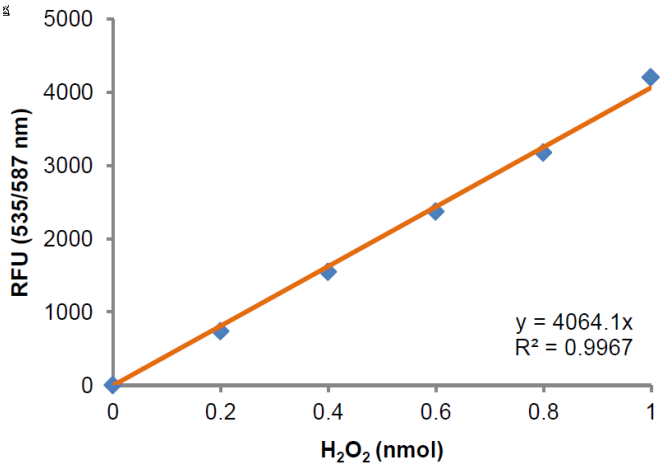


Figure 2.
Kinetic Measurement of DAO Activity from Positive Control.

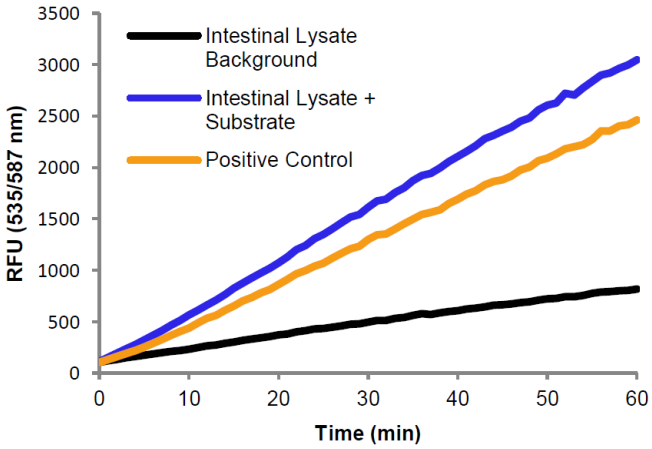
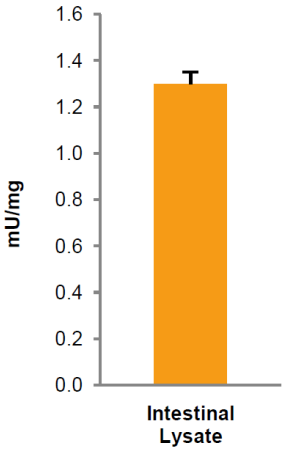


Figure 3.
Activity Determination of Intestinal Tissue Lysate.



For this experiment, 100 mg of rat intestine was used, following Diamine Oxidase Activity Assay Kit procedure 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.

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