

Version 1 Last updated 29 May 2020

# ab273325

## D-Amino Acid Oxidase Activity Assay Kit (Fluorometric)

View Kit datasheet: <https://www.abcam.com/ab273325>  
(use <https://www.abcam.cn/ab273325> for china, or  
<https://www.abcam.co.jp/ab273325> for Japan)

For the determination of DAAO activity in animal tissues.

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

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## 1. Overview

D-Amino Acid Oxidase Activity Assay Kit (Fluorometric) (ab273325) provides a quick and easy method for monitoring DAAO activities in a wide variety of samples. In this assay, DAAO converts D-amino acid into hydrogen peroxide as one byproduct. Consequently, the formed byproduct will react with a probe producing a strong fluorescent signal (Ex/Em= 535/587nm).

The kit is simple, sensitive, high-throughput adaptable and can detect as low as 0.5  $\mu$ U of DAAO activity.

## 2. Protocol Summary

Prepare all reagents, samples and positive controls.



Prepare standards.



Add all samples to the appropriate wells.



Add Reaction Mix and Background Control Mix to the appropriate wells.



Immediately measure fluorescence in kinetic mode for 30-45 mins, every minute at 25°C.



Determine DAAO activity using equation.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. Storage and Stability

**Store kit at -20°C in the dark immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
DAAO Assay Buffer	25 mL	-20°C
DAAO Substrate	220 µL	-20°C
DAAO Cofactor (lyophilized)	1 vial	-20°C
DAAO Probe (in DMSO)	200 µL	-20°C
DAAO Enzyme Mix (lyophilized)	1 vial	-20°C
H <sub>2</sub> O <sub>2</sub> Standard (0.88 M)	100 µL	-20°C
DAAO Positive Control (lyophilized)	1 vial	-20°C

## 7. Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Dounce Tissue Homogenizer
- 50% glycerol

## 8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

## 9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 9.1 **DAAO Assay Buffer:**

Store at -20 °C or 4 °C. Bring to room temperature before use.

### 9.2 **DAAO Substrate:**

Store at -20 °C or 4 °C. Bring to room temperature before use. Vortex contents until they are fully solubilized. No crystals should be seen.

### 9.3 **DAAO Cofactor:**

Reconstitute with 220 µl dH<sub>2</sub>O. Keep on ice while in use. Store at -20°C. Avoid light. Use within two months.

### 9.4 **DAAO Probe (in DMSO):**

Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Avoid light.

### 9.5 **DAAO Enzyme Mix:**

Reconstitute with 220 µl DAAO Assay buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw. Use within two months.

### 9.6 **H<sub>2</sub>O<sub>2</sub> Standard:**

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

### 9.7 **DAAO Positive Control:**

Reconstitute with 50 µl DAAO Assay Buffer and 50 µl 50% glycerol. Mix by pipetting up and down. Aliquot and store at -80°C. Avoid freeze and thaw. Keep on ice while in use. Use within two months.

## 10. Sample Preparation

### Tissue/cell lysate preparation:

- 10.1 Rapidly homogenize tissue (~10 mg) in 100  $\mu$ l ice cold DAAO Assay Buffer with Dounce Tissue Homogenizer and keep on ice for 10 mins.
- 10.2 Centrifuge at 10,000 x g for 5 mins. For lipid-rich samples, carefully avoid the lipid portion and transfer the supernatant to a fresh tube.
- 10.3 Add equal volume (2-50  $\mu$ l) of each sample into two wells (a background control is needed for each volume of unknown sample) of a 96-well clear plate and adjust the volume to 50  $\mu$ l with DAAO Assay Buffer.
- 10.4 **For DAAO positive control:** Take 5-10  $\mu$ l of DAAO Positive Control into desired well(s) and adjust the final volume to 50  $\mu$ l with DAAO Assay Buffer.

**Δ Note:** For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

**Δ Note:** For samples exhibiting significant background, prepare parallel sample well(s) as sample background controls.

## 11. Standard Curve

- 11.1 Add 10  $\mu\text{L}$  of the 0.88 M  $\text{H}_2\text{O}_2$  standard to 870  $\mu\text{L}$  of  $\text{dH}_2\text{O}$  to generate 10 mM  $\text{H}_2\text{O}_2$  standard.
- 11.2 Further dilute the 10 mM standard by adding 10  $\mu\text{L}$  of the 10 mM  $\text{H}_2\text{O}_2$  standard into 990  $\mu\text{L}$   $\text{dH}_2\text{O}$  to make a 0.1 mM  $\text{H}_2\text{O}_2$  standard. Mix well.
- 11.3 Add 0, 2, 4, 6, 8, 10  $\mu\text{L}$  of the 0.1 mM standards into a 96-well plate to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well. Bring volume to 50  $\mu\text{L}$  with the DAAO Assay buffer.
- 11.4 Do not store the diluted standards.

Standard #	0.1 mM $\text{H}_2\text{O}_2$ -Standard ( $\mu\text{L}$ )	DAAO Assay Buffer ( $\mu\text{L}$ )	$\text{H}_2\text{O}_2$ (nmol/well)
1	10	40	1.0
2	8	42	0.8
3	6	44	0.6
4	4	46	0.4
5	2	48	0.2
6	0	50	0

## 12. Assay Procedure

- Keep on ice during use

### Reaction mix:

- 12.1 Mix enough reagents for the number of assays to be performed.
- 12.2 Prepare a 5-fold dilution of DAAO Probe (e.g. Mix 5  $\mu$ l of DAAO Probe with 20  $\mu$ l DAAO Assay Buffer). For each well, prepare 50  $\mu$ l Mix containing:

	Reaction Mix ( $\mu$ l)	Background Mix ( $\mu$ l)
DAAO Assay Buffer	42	44
DAAO Substrate	2	---
DAAO Cofactor	2	2
Diluted DAAO Probe	2	2
DAAO Enzyme Mix	2	2

- 12.3 Mix and add 50  $\mu$ l of the Reaction Mix to each well containing the Standard, Positive Control and test samples.
- 12.4 Add 50  $\mu$ l of Background mix to the sample background control wells.
- 12.5 Measure Fluorescence (Ex/Em=535/587 nm) immediately in a microplate reader in kinetic mode for 30-45 mins and read every minute at 25°C.

## 13. Calculations

- 13.1 Subtract 0 Standard reading from all readings.
- 13.2 Plot the H<sub>2</sub>O<sub>2</sub>-Standard Curve and obtain the slope of the curve ( $\Delta\text{RFU}/\text{pmol}$ ).
- 13.3 If Sample Background Control reading is significant then subtract the Background Control reading from Sample readings.
- 13.4 To calculate the specific DAAO activity of Sample, subtract  $\Delta\text{RFU}$  of Negative Control ( $\Delta\text{RFU}_{\text{NC}}$ ) from Sample ( $\Delta\text{RFU}_{\text{S}}$ ).
- 13.5 Apply Sample  $\Delta\text{RFU}$  ( $\text{RFU}_2 - \text{RFU}_1$ ) to H<sub>2</sub>O<sub>2</sub> Standard Curve to get B nmol of product generated during the reaction time ( $\Delta t = t_2 - t_1$ ).

$$\text{Sample DAAO activity} = \frac{B}{(\Delta t \times V) \times D} \times P = \text{nmol/min/mg}$$

B = H<sub>2</sub>O<sub>2</sub> amount from Standard Curve (nmol)

$\Delta t$  = reaction time (mins)

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

D = Dilution Factor

P = Initial Sample Concentration in mg-protein/ml

### Unit definition:

One unit of D-amino acid oxidase is the amount of enzyme that generates 1.0  $\mu\text{mol}$  of H<sub>2</sub>O<sub>2</sub> per min at pH 8.3 at 25°C.

## 14. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

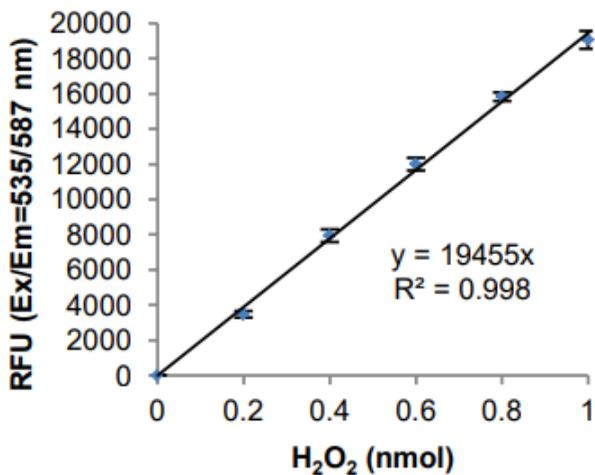


Figure 1. H<sub>2</sub>O<sub>2</sub> Standard curve.

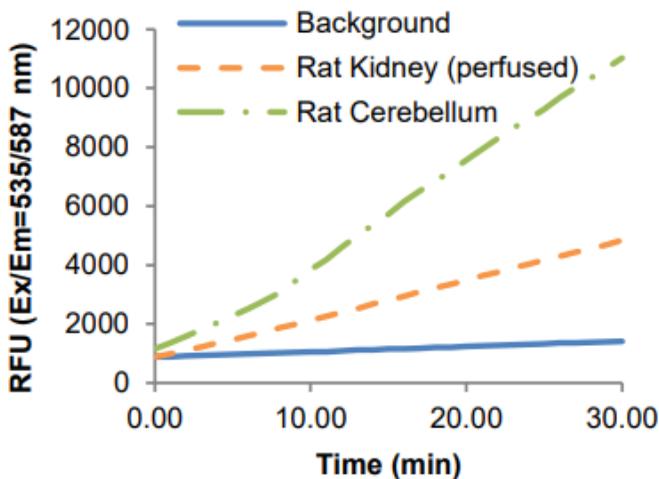
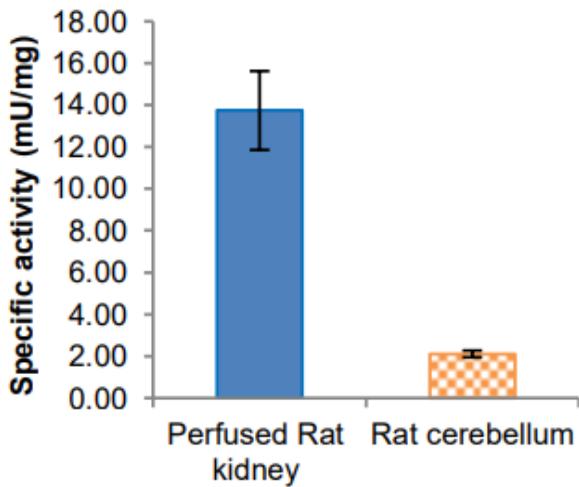


Figure 2. DAO activities in perfused rat kidney (0.447  $\mu$ g protein), rat cerebellum (8.67  $\mu$ g protein).



**Figure 3.** Specific DAAO activities in perfused rat kidney and rat cerebellum. Assays were performed following the kit protocol.

## 15. FAQ / Troubleshooting

General troubleshooting points are found at [www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines).

## 16. Notes

## Technical Support

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