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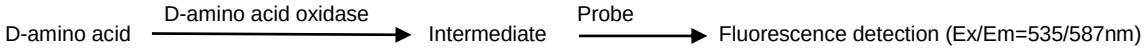
D-Amino Acid Oxidase Activity Assay Kit (Fluorometric)

(Catalog #K559-100; 100 assays; Store at -20°C)

I. Introduction:

D-amino acid oxidase (DAAO, DAO, EC 1.4.3.3) is a flavoprotein which oxidizes a D-amino acid forming hydrogen peroxide and the respective imino acid which in a second catalytic step by the same enzyme is hydrolyzed to a α-keto acid and ammonia. In recent years, DAAO has been studied for its possible role in schizophrenia. The simultaneous expression of DAAO and a putative product from G72 gene may potentially increase the risk of schizophrenia in patients. For example, increased DAAO expression and enzymatic activity have been reported in post mortem brain tissue samples from patients with schizophrenia compared to healthy controls. DAAO expression in human brain is very specific: it can only be found in the cerebellum and is not detectable in any other parts of the brain. DAAO regulates the physiological levels of D-amino acids such as D-serine, D-aspartate and D-alanine. D-serine acts as a modulator of *N*-Methyl-D-aspartate receptor (NMDA receptor, NMDAR), while D-aspartate and D-alanine are found to be elevated in both white matter and gray matter in patients' brains suffering from Alzheimer disease. BioVision's D-amino Acid Oxidase Activity Assay Kit 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 µU of DAAO activity.



II. Applications:

- Measurement of D-amino acid oxidase activity in various tissues
- Analysis of correlation of D-amino acid oxidase with schizophrenia, Alzheimer disease, etc.

III. Sample Type:

- Animal tissues: kidney, cerebellum, etc.

IV. Kit Contents:

Components	K559-100	Cap Code	Part Number
DAAO Assay Buffer	25 ml	WM	K559-100-1
DAAO Substrate	220 µl	Blue	K559-100-2
DAAO Cofactor	1 vial	Brown	K559-100-3
DAAO Probe (in DMSO)	200 µl	Red	K559-100-4
DAAO Enzyme Mix	1 vial	Green	K559-100-5
H_2O_2 Standard (0.88 M)	100 µl	Yellow	K559-100-6
DAAO Positive Control	1 vial	Orange	K559-100-7

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Dounce Tissue Homogenizer (Cat. #1998)
- 50% glycerol

VI. Storage Conditions and Reagent Preparation:

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

- **DAAO Assay Buffer:** Warm to room temperature before use. Store at 4ºC or -20ºC.
- **DAAO Substrate:** Warm to room temperature before use. Vortex contents until they are fully solubilized. *No crystals should be seen*. Store at -20°C. Use within two months.
- **DAAO Cofactor:** Reconstitute with 220 µl dH_2O. Keep on ice while in use. Store at -20°C. Avoid light. Use within two months.
- **DAAO Probe (in DMSO):** Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Avoid light.
- **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.
- **H_2O_2 Standard:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **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.

VII. D-Amino Acid Oxidase Assay Protocol:

1. **Sample Preparation:** Rapidly homogenize tissue (~10 mg) in 100 µl ice cold DAAO Assay Buffer with Dounce Tissue Homogenizer (Cat. #1998), and keep on ice for 10 min. Centrifuge at 10,000 x g for 5 min. For lipid-rich samples, carefully avoid the lipid portion and transfer the supernatant to a fresh tube. Add equal volume (2-50 µl) 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 µl with DAAO Assay Buffer. **For DAAO positive control:** Take 5-10 µl of DAAO Positive Control into desired well(s) and adjust the final volume to 50 µl with DAAO Assay Buffer.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

b. For samples exhibiting significant background, prepare parallel sample well(s) as sample background controls.

2. **Standard Curve Preparation:** Add 10 µl of the 0.88 M H_2O_2 standard to 870 µl of dH_2O to generate 10 mM H_2O_2 standard. Further dilute the 10 mM standard by adding 10 µl of the 10 mM H_2O_2 standard into 990 µl dH_2O to make a 0.1 mM H_2O_2 standard. Mix well. Add 0, 2, 4, 6, 8, 10 µl 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 µl with the DAAO Assay buffer. ***Do not store the diluted standards*.**
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Prepare a 5-fold dilution of DAAO Probe (e.g. Mix 5 µl of DAAO Probe with 20 µl DAAO Assay Buffer). For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Mix
DAAO Assay Buffer	42 µl	44 µl
DAAO Substrate	2 µl	----
DAAO Cofactor	2 µl	2 µl
Diluted DAAO Probe	2 µl	2 µl
DAAO Enzyme Mix	2 µl	2 µl

Mix and add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples. Add 50 µl of Background mix to the sample background control wells.

4. **Measurement:** Measure Fluorescence (Ex/Em=535/587 nm) immediately in a microplate reader in kinetic mode for 30-45 min and read every minute at 25°C.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the H_2O_2 Standard Curve. Subtract the sample background control reading from its paired sample reading. Select the linear portion of the kinetic curve for DAAO activity calculation. Apply Sample ΔRFU ($RFU_2 – RFU_1$) to H_2O_2 Standard Curve to get B nmol of product generated during the reaction time ($\Delta t = t_2 - t_1$).

Sample DAAO Activity = B/[Δt X V]*D x P = nmol/min/mg = mU/mg

Where: **B** = H_2O_2 amount from Standard Curve (nmol)
Δt = reaction time (min)
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 µmol of H_2O_2 per min at pH 8.3 at 25°C.

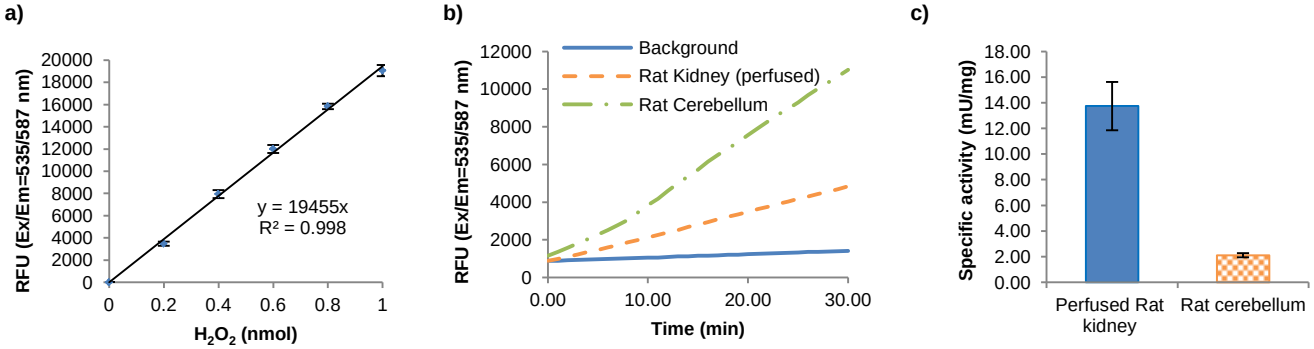


Figure: (a) H_2O_2 Standard curve; (b) DAAO activities in perfused Rat kidney (0.447 µg protein), Rat cerebellum (8.67 µg protein); (c) Specific DAAO activities in Perfused Rat kidney and Rat cerebellum. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

D-amino acid oxidase, human recombinant (P1052)
Pimavanserin Tartrate (B1915)
FAD Colorimetric/Fluorometric Assay Kit (K357)
Ceruloplasmin Activity Colorimetric Assay Kit (K669)
Mutant Alanine Racemase Y354N, Active, Recombinant (P116)
Dextromethrophan (hydrobromide hydrate) (2912)
DL-serine Assay Kit (Fluorometric) (K545)
Glyoxalase I Activity Assay Kit (Colorimetric) (K591)
BRD1 bromodomain (His-Tag), human recombinant (7405)
Phospho (Tyr1472) NMDA NR2B Antibody (3616)
Anti-Alanine Racemase Antibody (A1380)
D (+)-Cycloserine (B1188)

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