Product Manual

D-Amino Acid Assay Kit (Fluorometric)

Catalog Number

MET-5137 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

D-Amino acids are amino acids where the stereogenic carbon, or chiral carbon, next to the amino group has the D-configuration. "D" stands for dextrorotary, meaning that the isomer rotates plane polarized light clockwise. For most naturally-occurring amino acids, this carbon has the L-configuration, although D-Amino acids are sometimes found as residues in proteins.

D-Amino acids have been found in various foods such as fruits, vegetables, milk, cheese, meats, and fish. While D-amino acids are not detected in human serum, micromolar levels can be detected in the mammalian brain and endocrine systems of humans and mice. For example, D-serine is released from neurons, through alanine-serine-cysteine transporter-1 (Asc-1) or other pathways, into the synapse where it can bind and affect NMDA receptor activity. D-serine induces long term potentiation (LTP, a model of learning and memory), and breakdown of D-serine by D-amino acid oxidase causes suppression of LTP induction. Aberrant levels of D-serine are associated with diseases caused by abnormal NMDA receptor activity. Increased levels of D-serine in the cerebrospinal fluid (CSF) have been detected in patients with Alzheimer's disease compared to normal controls. Decreased D-serine levels (which lowers NMDA receptor signaling) is associated with schizophrenia-like symptoms.

Cell Biolabs' D-Amino Acid Assay Kit (Fluorometric) is a simple fluorometric assay that measures the total amount of free D-Amino Acids present in biological samples (except for D-glutamate, D-aspartate and glycine) in a 96-well microtiter plate format. Amino Acids found in polypeptide chains (peptides and proteins) are not detected. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, standards and unknown samples. Sample D-Amino Acid concentrations are determined by comparison with a known D-Alanine standard. The kit has a detection sensitivity limit of 1.56 µM D-Amino Acids.

Assay Principle

Cell Biolabs' D-Amino Acid Assay Kit (Fluorometric) measures free D-Amino Acids (except for D-Glutamate, D-Aspartate and Glycine) present in foods or biological samples in a 96-well microtiter plate format. D-amino acids are oxidatively deaminated by D-Amino Acid oxidase into the corresponding α-keto acid, ammonia, and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are read with a standard 96-well fluorometric plate reader. Samples are compared to a known concentration of D-Alanine standard within the 96-well microtiter plate format (Figure 1).

Note: Each sample replicate requires 2 assays, one treated with D-Amino Acid oxidase (+DAAO) and one without (-DAAO). D-Amino Acid content is calculated from the difference in OD readings from the 2 wells.

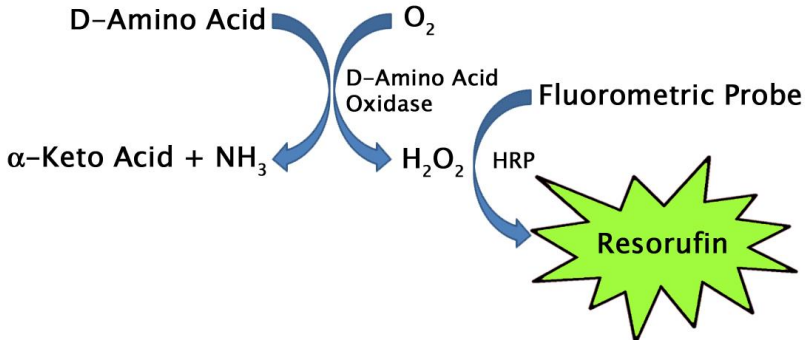

Figure 1. D-Amino Acid Assay Principle.

Related Products

1. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
2. MET-5070: Glycine Assay Kit
3. STA-674: Glutamate Assay Kit (Fluorometric)
4. MET-5093: Alanine Assay Kit
5. MET-5129: Lysine Assay Kit (Fluorometric)

Kit Components

1. D-Alanine Standard (Part No. 51361C): One 50 µL tube at 10 mM.
2. 10X Assay Buffer (Part No. 50802A): One 30 mL bottle.
3. Fluorometric Probe (Part No. 50231C): One 50 µL amber tube.
4. HRP (Part No. 234402): One 100 µL tube at 100 U/mL in glycerol.
5. D-Amino Acid Oxidase (Part No. 51362D): One 250 µL tube containing D-Amino Acid Oxidase from Porcine Kidney.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Standard 96-well black microtiter plate and/or cell culture microplate

Storage

Upon receipt, store the 10X Assay Buffer at room temperature. Store the D-Amino Acid Oxidase at -80ºC. Store all other components at -20ºC. The Fluorometric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer to 1X with deionized water. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, and D-Amino Acid Oxidase 1:20 in 1X Assay Buffer. For example, add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution, and 50 µL of D-Amino Acid Oxidase to 938 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays.

Note: Prepare only enough for immediate use by scaling the above example proportionally.

- Control Mix: Prepare a Control mix by diluting the Fluorometric Probe1:100 and HRP 1:500 in 1X Assay Buffer. For example, add 10 µL of Fluorometric Probe and 2 µL of HRP to 988 µL of 1X Assay Buffer for a total of 1 mL. This Control Mix volume is enough for 20 assays.

Note: Scale the described example up or down appropriately and prepare only enough for immediate use.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorometric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh D-Alanine Standards before use by diluting in 1X Assay Buffer according to Table 2.

Standard Tubes	10 mM D-Alanine Solution (µL)	1X Assay Buffer (µL)	D-Alanine (µM)
1	5	495	100
2	250 of Tube #1	250	50
3	250 of Tube #2	250	25
4	250 of Tube #3	250	12.5
5	250 of Tube #4	250	6.25
6	250 of Tube #5	250	3.13
7	250 of Tube #6	250	1.56
8	0	250	0

Table 2. Preparation of D-Alanine Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

Note: Each sample replicate requires two paired wells, one to be treated with D-Amino Acid Oxidase (Reaction Mix) and one without the enzyme (Control Mix) to measure endogenous sample background.

2. Add 50 µL of each D-Alanine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 50 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate for 1 hour at 37°C protected from light.

Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.

6. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.

Example of Results

The following figures demonstrate typical D-Amino Acid Assay Kit (Fluorometric) results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

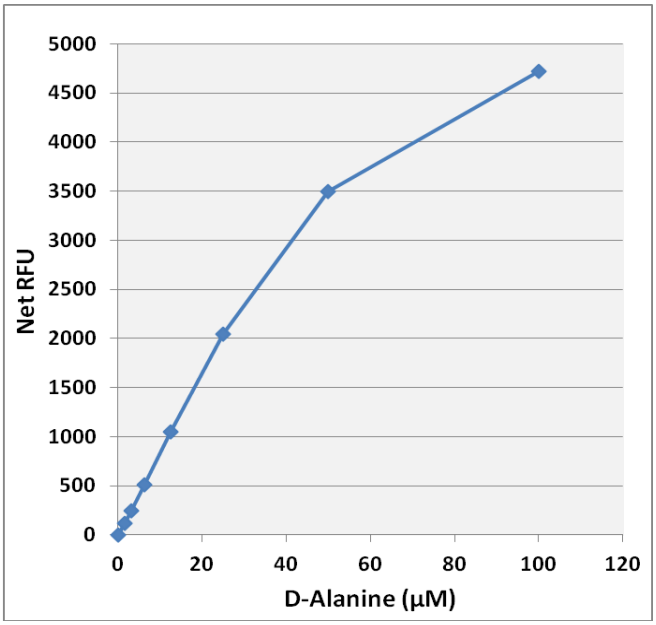

Figure 2: D-Alanine Standard Curve.

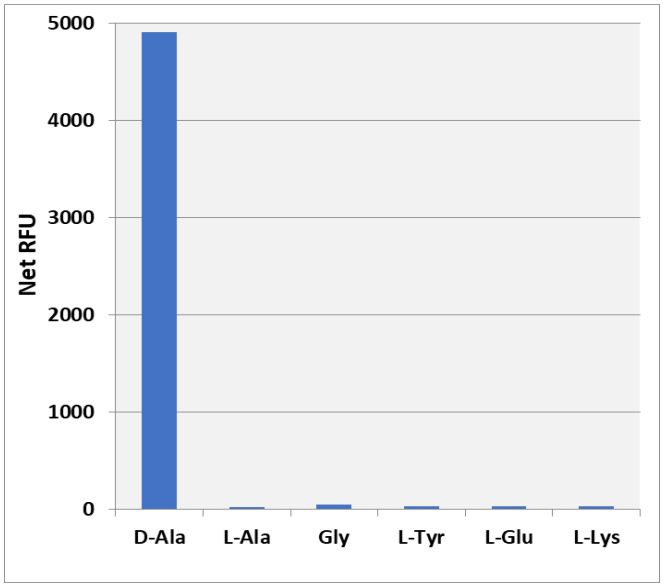

Figure 3: D-Amino Acid Oxidase specificity. D-Alanine, L-alanine, glycine, L-Tyrosine, L-Glutamate, or L-Lysine was tested at a concentration of 100 µM according to the assay protocol.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.

2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without D-Amino Acid Oxidase (-DAAO) from the sample well values containing enzyme (+DAAO) to obtain the difference. The absorbance difference is due to the D-Amino Acid Oxidase activity:

$$\Delta RFU = (RFU_{+DAAO}) - (RFU_{-DAAO})$$

5. Compare the ΔRFU of each sample to the standard curve to determine and extrapolate the quantity of D-Amino Acids present in the sample. Only use values within the range of the standard curve.

References

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