Version 1 Last updated 28 September 2018

ab239721 D-Amino Acid Assay Kit (Fluorometric)

For the measurement of total D-amino acids in tissues and biological fluids.

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

Table of Contents

1. Overview

D-Amino Acid Assay Kit (Fluorometric) (ab239721) provides a quick, specific and easy method for measuring total D-Amino acid concentrations in a wide variety of samples. In this assay, D-Amino acids are converted into an intermediate by the DAA enzyme mix that will further react with a probe to produce a strong fluorescence signal (Ex/Em= 535/587nm). The kit is simple to use, sensitive and high-throughput adaptable and can detect as low as 1.9 µM of D-Amino acids in biological samples.

2. Protocol Summary

Prepare tissue / biological fluid samples (Sample, Sample background and Spike).

Prepare Standard Curve dilutions, Reaction Mix and Background Reaction Mix.

Add Standards, Sample, Sample background and Spike to the plate and bring volume to 50 µl with DAA Assay Buffer.

Add 50 µl of Reaction Mix or Background Reaction Mix to the appropriate wells.

Incubate at 37°C for 2 hours protected from light.

Measure fluorescence (Ex/Em = 535/587 nm) in a microplate reader

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 on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
DAA Assay Buffer	25 mL	4°C or -20°C	4°C or -20°C
DAA Cofactor	1 vial	-20°C	-20°C
DAA Enzyme Mix	1 vial	-20°C	-20°C
DAA Developer Mix	1 vial	-20°C	-20°C
DAA Probe	200 µl	-20°C	-20°C
DAA Standard	1 vial	-20°C	-20°C

5. 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
- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- Dounce tissue homogenizer

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 DAA Assay Buffer

Store at -20°C or 4°C. Bring to room temperature (RT) before use. Ready to use as supplied.

6.2 DAA Cofactor

Reconstitute with 220 µl dH2O. Keep on ice while in use. Store at -20°C. Avoid light. Use within two months.

6.3 DAA Enzyme Mix

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

6.4 DAA Developer Mix

Reconstitute each vial with 220 µl DAA Assay buffer. Aliquot and store at -20°C. Keep on ice

while in use. Avoid freeze and thaw. Use within two months.

6.5 DAA Probe

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

6.6 DAA Standard

Reconstitute with 110 µl of dH2O to make a 10 mM stock solution. Store at -20°C, stable for 5 freeze/thaw cycles.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Prepare 100 µM solution of D-Amino Acid Standard by adding 10 µL of the 10 mM D-Amino acid standard stock to 990 µl of dH_2O.

7.2 Add 0, 2, 4, 6, 8, and 10 µL of 100 µM D-Amino Acid Standard into a series of wells in a 96-well plate to generate 0, 200, 400, 600, 800 and 1,000 pmol of D-Amino Acid Standard/well. Adjust the volume to 50 µL/well with DAA Assay Buffer.

Standard #	100 µM D-Amino Acid Standard (µL)	DAA Assay Buffer (µL)	Final volume standard in well (µL)	D-Amino Acid (pmol/well)
1	20	80	100	1000
2	16	84	100	800
3	12	88	100	600
4	8	92	100	400
5	4	96	100	200
6	0	100	100	0

Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

8. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

8.1 Tissue samples:

8.1.1 Rapidly homogenize tissue (~10 mg) in 100 µL ice cold DAA Assay Buffer with Dounce tissue homogenizer and keep on ice for 10 minutes. Centrifuge at 10,000 x g for 5 minutes at 4 °C.

8.1.2 For lipid-rich samples, carefully avoid/remove the lipid-rich portion and transfer the aqueous phase (supernatant) to a 10kDa MW cut-off spin column. Centrifuge the sample at 10,000 x g for 10 minutes at 4 °C and collect the filtrate.

8.2 Biological fluids:

8.2.1 Add 200-500 µL of sample into a 10kDa MW cut-off spin column. Centrifuge the sample at 10,000 x g for 10 minutes at 4 °C and collect the filtrate.

All sample types:

Due to matrix effect in biological samples, an internal standard (spiking) is needed for each sample.

1. For each test sample, prepare 3 parallel sample wells. Add 2-50 Ll of samples (2-10 µL of brain tissue sample and 5-25 µL of CSF) into 3 wells in a 96-well clear plate. Label each well as "Sample", "Sample Background", "Spike".
2. Dilute D-Amino acid standard to 100 µM by adding 10 µL of the 10 mM stock solution into 990 µl of dH2O. Add 2-5 µL of the 0.1 mM D-Amino acid standard into the "spike" wells. Bring the volume of all the wells to 50 µL with DAA Assay buffer. For unknown samples, prepare parallel wells with different dilutions.

9. Assay Procedure

– Assay all standards, controls and samples in duplicate.

9.1 Dilute DAA Probe 5-fold (i.e. 5 µL DAA Probe + 20 µL DAA Assay Buffer).

9.2 Prepare 50 µL of Reaction Mix and Background Mix for each reaction. Prepare a master mix to ensure consistency. Mix and add 50 µl of the Reaction Mix to each well containing the Standard, Sample and Spike wells. Add 50 µl of Background Mix to the Sample Background wells.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
DAA Assay Buffer	42	44
DAA Cofactor	2	2
DAA Enzyme Mix	2	-
DAA Developer Mix	2	2
Diluted DAA Probe	2	2

9.3 Incubate at 37°C for 2 hours. Protect from light. Measure fluorescence (Ex/Em = 535/587 nm) in a microplate reader.

10. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract 0 Standard reading from all standard readings.
3. Plot the D-Amino Acid Standard Curve.
4. Subtract Sample background reading from Sample reading (F_S = RFU_S – RFU_{sbc}) and subtract Sample background reading from Spike reading (F_{spike} =RFU_{spike} – RFU_{sbc}).

$$DAA\ in\ sample\ wells\ (B) = \frac{\mathrm{Fs}}{\mathrm{Fspike - Fs}} * \mathrm{D}$$ -Amino acid spike (in pmol)

For biological fluids:

$$DAA\ in\ sample\ = \frac{\mathrm{B}}{\mathrm{V}} * \mathrm{D} =$$ pmol/µL = µM

Where:

B = amount of D-Amino Acid in sample well (pmol.)

V = sample volume added in the sample wells (µL).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up; D=1 for undiluted samples).

For tissue samples:

$$DAA\ in\ sample\ = \frac{\mathrm{B}}{\mathrm{V}} * \mathrm{D} * \mathrm{P} =$$ pmol/µg

Where:

B = amount of D-Amino Acid in sample well (pmol.)

V = sample volume added in the sample wells (µL).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up; D=1 for undiluted samples).

P = sample protein concentration the untreated samples (µg protein/µL).

11. Typical Data

Data provided **for demonstration purposes only.**

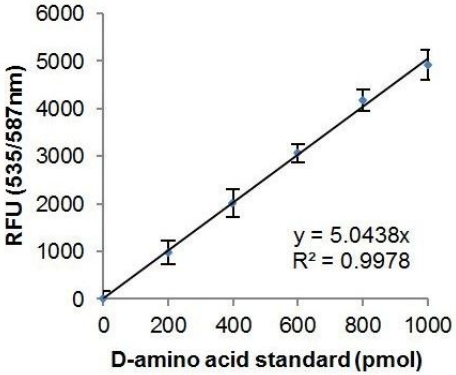

Figure 1. D-Amino Acid standard curve.

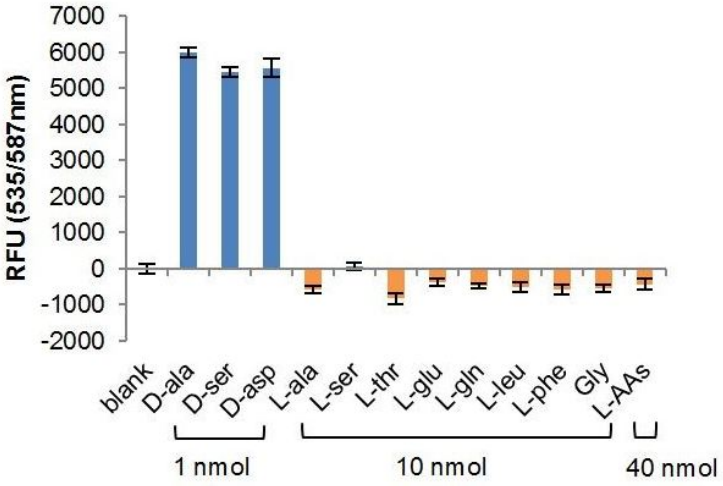

Figure 2. Assay specificity: L-Amino acids were tested at a 10-fold molar excess (10 nmol each) and 40-fold molar excess for a mixture od L-amino acids (40 nmol total).

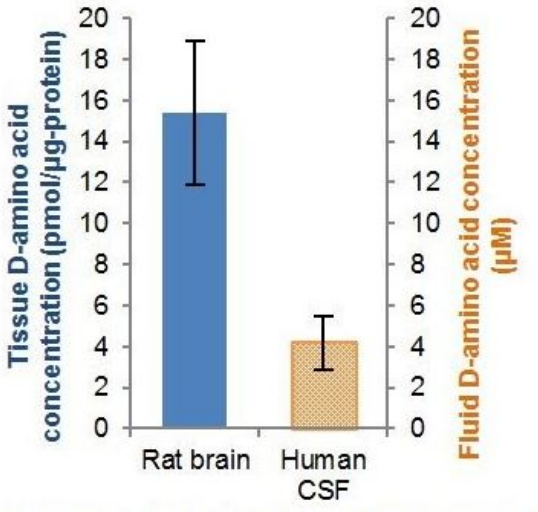

Figure 3. Estimations of total D-Amino acids in rat brain samples and pooled normal human CSF (15 µl). D-Amino acid concentrations were 15.4 pmol/µg-protein in rat brain and 4.2 µM in human CSF.

12. Notes

Technical Support

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