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Total D-Amino Acid Assay Kit (Fluorometric)

05/18

(Catalog #K445-100; 100 assays; Store at -20°C)

I. Introduction:

D-Amino acids are present in both eukaryotes and prokaryotes. D-Amino acids act as a component of the cell wall in prokaryotes and a neurological regulator in eukaryotes. In mammals, D-Amino acids play an important role in physiological functions and their levels are regulated by D-Amino acid oxidase in the central nervous systems. Some important D-Amino acids include 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 have been found to be elevated in the white and the gray matter respectively in patients' brains suffering Alzheimer's disease. The abnormal levels of D-Amino acids could be used as a marker of neurological diseases: total D-Amino acid concentration in cerebrospinal fluid (CSF) has been shown to be higher in patients with Alzheimer's disease when compared to samples from healthy donors. BioVision's Total D-Amino Acid Assay Kit (Fluorometric) 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.

D-Amino acids —(DAA Enzyme Mix)→ Intermediate —(Probe)→ Fluorescence detection (Ex/Em=535/587nm)

II. Applications:

- Measurement of total D-Amino acids concentrations in biological samples
- Analysis of relationship between D-Amino acids in physiological conditions including schizophrenia, Alzheimer disease, etc.

III. Sample Type:

- Biological fluids: cerebrospinal fluid, etc.
- Animal tissues: brain, etc.

IV. Kit Contents:

Components	K445-100	Cap Code	Part Number
DAA Assay Buffer	25 ml	WM	K445-100-1
DAA Cofactor	1 vial	Brown	K445-100-2
DAA Enzyme Mix	1 vial	Blue	K445-100-3
DAA Developer Mix	1 vial	Green	K445-100-4
DAA Probe	200 µl	Red	K445-100-5
DAA Standard	1 vial	Yellow	K445-100-6

V. User Supplied Reagents and Equipment:

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

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.

- **DAA Assay Buffer:** Warm to room temperature before use. Store at 4ºC or -20ºC.
- **DAA Cofactor:** Reconstitute with 220 µl dH_2O. Keep on ice while in use. Store at -20°C. Avoid light. Use within two months.
- **DAA Enzyme Mix, 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.
- **DAA Probe (in DMSO):** Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Avoid excessive exposure to light.
- **D-Amino acid Standard:** Reconstitute with 110 µl of dH_2O to make a 10 mM stock solution. Store at -20°C, stable for 5 freeze/thaw cycles.

VII. D-Amino Acid Oxidase Assay Protocol:

1. **Sample Preparation: For tissue samples:** 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 at 4 °C. For lipid-rich samples, carefully avoid/remove the lipid-rich portion and transfer the aqueous phase (supernatant) to a 10kDa MWCO Spin Columns (Cat. # 1997). Centrifuge the sample at 10,000 *x g* for 10 min at 4 °C and collect the filtrate. **For biological fluids:** Add 200-500 µl of sample into a 10kDa MWCO Spin Columns (Cat. # 1997). Centrifuge the sample at 10,000 *x g* for 10 min at 4 °C and collect the filtrate. **For all samples:** Due to matrix effect in biological samples, an internal standard (spiking) is needed for each sample. For each test sample, prepare 3 parallel sample wells. Add 2-50 µl 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". Dilute D-Amino acid standard to 100 µM by adding 10 µl of the 10 mM stock solution into 990 µl of dH_2O. 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.

2. **Standard Curve Preparation:** Prepare a 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. Add 0, 2, 4, 6, 8, 10 µl of the 100 µM working D-Amino acid standard into a series of wells, generating 0, 200, 400, 600, 800, 1000 pmol of D-Amino acid/well. Adjust the volume to 50 µl/well with DAA Assay buffer
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Prepare a 5-fold dilution of DAA Probe (e.g. Mix 5 µl of DAA Probe with 20 µl DAA Assay Buffer). For each well, prepare 50 µl Mix containing:

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

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.

4. **Measurement:** Incubate at 37°C for 2 hours. Protect from light. Measure fluorescence (Ex/Em = 535/587 nm) in a microplate reader.
5. **Calculation:** Subtract 0 Standard reading from all standard readings. Plot the D-Amino acid Standard Curve. 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}).

Amount of D-Amino acids in sample wells (B) = $\frac{F_s}{F_{spike}-F_s}$ ×D-Amino acid Spike (in pmol)

For biological fluids: Sample D-Amino acid Concentration = $\frac{B}{V} \times D$ = pmol/ µl = µM

Where: V is the volume of sample added to the well (in µl)
D is the sample dilution factor (if applicable, D=1 for undiluted samples)

For tissue samples: Sample D-Amino acid Concentration = $\frac{B}{V} \times D \times P$ = pmol/ µg

Where: V is the volume of sample added to the well (in µl)
D is the sample dilution factor (if applicable, D=1 for undiluted samples)
P is the sample protein concentration in the untreated samples (µg-protein/µl)

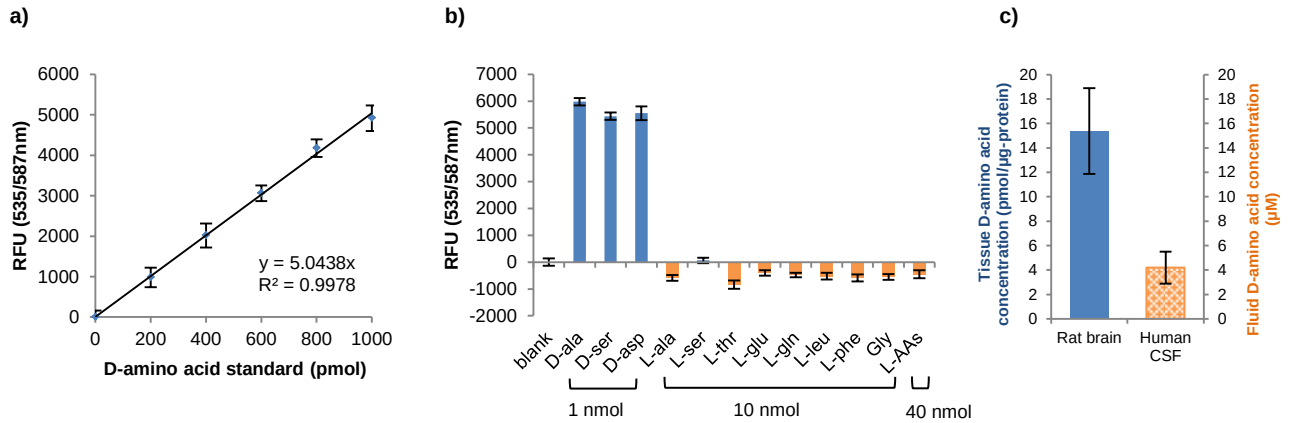

Figure: (a) D-Amino acid standard curve; (b) Specificity of the assay: L-AAs were tested at a 10-fold molar excess (each AA: 10 nmol) and 40-fold excess of a mixture of L-AAs (Total: 40 nmol) vs D-AAs (each D-AA: 1 nmol). (c) Estimations of total D-Amino acids in rat brain sample and pooled normal human CSF (15 µl). D-Amino acid concentrations were 15.4 pmol/µg-protein in rat brain and 4.2 µM in Human CSF. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

DL-serine Assay Kit (Fluorometric) (K545)
Pimavanserin Tartrate (B1915)
FAD Colorimetric/Fluorometric Assay Kit (7645)
Ceruloplasmin Activity Colorimetric Assay Kit (K669)
Mutant Alanine Racemase Y354N, Active, Recombinant (P116)
Dextromethrophan (hydrobromide hydrate) (2912)
D-Amino acid oxidase, human recombinant (P1052)
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)

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