EnzyChrom™ D-Amino Acid Assay Kit (EDAA-100)

Quantitative Colorimetric and Fluorimetric D-Amino Acid Determination

DESCRIPTION

D-AMINO-ACIDS are not as widespread as their enantiomeric counterparts in proteins but they can be found in organisms ranging from bacteria (cell walls and antibiotics) to mammals (central nervous systems). The presence of D-amino acids in food is also of considerable interest. Racemization of L-amino acids during food processing may affect food quality and nutritional value.

BioAssay Systems' D-amino acid assay uses an enzyme-catalyzed oxidation of D-amino acids to convert a dye into a colored and fluorescent form. The absorbance at 570 nm or fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm is directly proportional to the D-amino acid concentration in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range: 0.86 to 500 µM (colorimetric assay) and 0.18 to 50 µM (fluorimetric assay) for 60 min reaction.

Convenient. The procedure involves adding a single working reagent and reading after 60 minutes.

High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to process thousands of samples per day.

APPLICATIONS

D-Amino Acid determination in tissue, milk, and other biological preparations.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	12 mL	**HRP Enzyme:**	120 µL
DAA Enzyme:	120 µL	**Dye Reagent:**	120 µL
Standard:	500 µL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Safety Data Sheet for detailed information.

PROCEDURES

Important: this assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough.

Sample Preparation

Tissue samples can be prepared by homogenizing 20-100 mg in 200 - 1000 µL dH_2O. Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

Milk samples often require at least 2× dilution in the Assay Buffer.

Reagent Preparation

Equilibrate all reagents to room temperature. Briefly centrifuge tubes before opening.

Colorimetric Procedure

1. *Standards*. Prepare 200 µL of 500 µM Premix by mixing 50 µL of the Standard (2 mM) and 150 µL dH_2O. Dilute standards in 1.5-mL centrifuge tubes as described in the Table. Transfer 20 µL Standards into separate wells of a *clear flat bottom 96-well plate*.

No.	Premix + H_2O	Standard (µM)
1	100 µL + 0 µL	500
2	60 µL + 40 µL	300
3	30 µL + 70 µL	150
4	0 µL + 100 µL	0

2. Transfer 20 µL of each sample into separate wells.
3. Prepare enough Working Reagent (WR) for all assay wells by mixing, for each well, 85 µL Assay Buffer, 1 µL DAA Enzyme, 1 µL HRP Enzyme, and 1 µL Dye Reagent.
4. Add 80 µL of the WR to each well. Tap plate briefly to mix.
5. Incubate at room temperature for 60 min. Use a plate reader to measure OD_{570nm}.

Fluorimetric Procedure

Dilute the standards prepared in Colorimetric Procedure 1:10 in dH_2O.

Transfer 20 µL standards and 20 µL samples into separate wells of a *black flat bottom 96-well plate*.

Add 80 µL of the Working Reagent to each well (see Colorimetric Procedure step 3). Tap plate to mix.

Incubate protected from light for 60 min at RT and measure fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

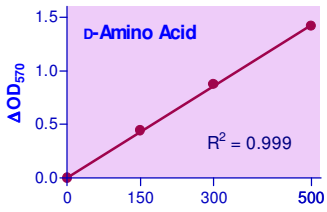
Subtract blank value (water, standard #4) from the standard values and plot the adjusted values against standard concentrations. Determine the slope and calculate the D-amino acid concentration of Sample as follows

$$[\text{D-Amino Acid}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

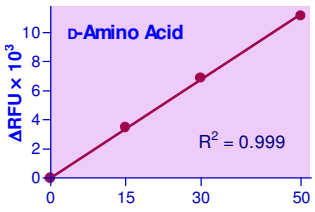
where R_{SAMPLE} and R_{BLANK} are the OD or fluorescence values of the Sample and H_2O Blank, respectively. *n* is the sample dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor, pipette tips, etc), clear (colorimetric) or black (fluorimetric) flat-bottom 96-well plates, centrifuge tubes, and plate reader.



96-well colorimetric assay



96-well fluorimetric assay

LITERATURE

1. Friedman, M. (1999) Chemistry, nutrition, and microbiology of D-amino acids. J Agric Food Chem. 47(9):3457-79.
2. Fuchs, S.A., et al. (2005) D-amino acids in the central nervous system in health and disease. Mol Genet Metab. 85(3):168-80.
3. Molla, G., et al. (2012) Enzymatic detection of D-amino acids. Methods Mol Biol. 794:273-89.

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