EnzyChrom™ L-Amino Acid Assay Kit (ELAA-100)

Quantitative Colorimetric and Fluorimetric L-Amino Acid Determination

DESCRIPTION

L-AMINO-ACIDS are the building blocks of proteins in biology. Almost all of the common 20 amino acids exist as the L-enantiomer. BioAssay Systems' L-amino acid assay uses an enzyme-catalyzed oxidation of L-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 L-amino acid concentration in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range: 3.3 to 500 µM (colorimetric assay) and 0.13 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

L-Amino Acid determination in serum, culture media, tissue homogenates, cell lysates, urine, food samples, etc.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 12 mL **HRP Enzyme:** 120 µL
LAA Enzyme: 120 µL **Dye Reagent:** 120 µL
Standard: 1 mL

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

Sample Preparation

Tissue or solid samples (e.g. food): Homogenize 20-100 mg Sample in 200 - 1000 µL dH_2O. Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 14,000 × g for 10 min at 4°C. Remove supernatant for assay.

Liquid Samples can be assayed directly. It is recommended to dilute serum and cell culture media samples 4-fold in dH_2O. For urine samples, use an internal standard method (see Product FAQ on our website).

Reagent Preparation

Equilibrate all reagents to room temperature.

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 LAA Enzyme, 1 µL HRP Enzyme, 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 read 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 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 read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

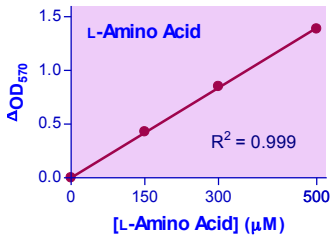
Subtract blank value (water, standard #4) from the standard values and plot the adjusted values against standard concentrations. Determine the slope and calculate the L-amino acid concentration of Sample as follows

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

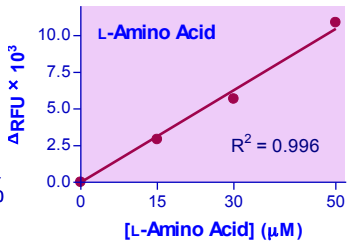
where R_{SAMPLE} and R_{BLANK} are the OD or fluorescence values of the Sample and H_2O Blank, respectively. *n* is the sample dilution factor.

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. Fisher, G., et al (1998) Free D- and L-amino acids in ventricular cerebrospinal fluid from Alzheimer and normal subjects. Amino Acids. 1998;15(3):263-9.
2. Váradi, M., et al (1999) Determination of the ratio of d- and l-amino acids in brewing by an immobilised amino acid oxidase enzyme reactor coupled to amperometric detection. Biosens Bioelectron. 1999 Mar 15;14(3):335-40.
3. Amelung, W., et al (2001) Determination of amino acid enantiomers in soil. Soil Biology and Biochemistry. 2001 Apr 1;15(4-5):553-562.