

## 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  $\lambda_{\text{ex/em}} = 530/585$  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  $\mu\text{M}$  (colorimetric assay) and 0.13 to 50  $\mu\text{M}$  (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  $\mu\text{L}$   
**LAA Enzyme:** 120  $\mu\text{L}$                       **Dye Reagent:** 120  $\mu\text{L}$   
**Standard:** 1 mL

**Storage conditions.** The kit is shipped on ice. Store all components at  $-20^\circ\text{C}$  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  $\mu\text{L}$   $\text{dH}_2\text{O}$ . Centrifuge at  $10,000 \times g$  for 15 min at  $4^\circ\text{C}$ . Remove supernatant for assay.

**Cell Lysate:** Collect cells by centrifugation at  $2,000 \times g$  for 5 min at  $4^\circ\text{C}$ . 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  $14,000 \times g$  for 10 min at  $4^\circ\text{C}$ . Remove supernatant for assay.

**Liquid Samples** can be assayed directly. It is recommended to dilute serum and cell culture media samples 4-fold in  $\text{dH}_2\text{O}$ . For urine samples, use an internal standard method (see Product FAQ on our website).

##### Reagent Preparation

Equilibrate all reagents to room temperature.

##### Colorimetric Procedure

- Standards.** Prepare 200  $\mu\text{L}$  of 500  $\mu\text{M}$  Premix by mixing 50  $\mu\text{L}$  of the Standard (2 mM) and 150  $\mu\text{L}$   $\text{dH}_2\text{O}$ . Dilute standards in 1.5-mL centrifuge tubes as described in the Table. Transfer 20  $\mu\text{L}$  Standards into separate wells of a *clear flat bottom 96-well plate*.

No	Premix + $\text{H}_2\text{O}$	Standard ( $\mu\text{M}$ )
1	100 $\mu\text{L}$ + 0 $\mu\text{L}$	500
2	60 $\mu\text{L}$ + 40 $\mu\text{L}$	300
3	30 $\mu\text{L}$ + 70 $\mu\text{L}$	150
4	0 $\mu\text{L}$ + 100 $\mu\text{L}$	0

- Transfer 20  $\mu\text{L}$  of each sample into separate wells.
- Prepare enough Working Reagent (WR) for all assay wells by mixing, for each well, 85  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  LAA Enzyme, 1  $\mu\text{L}$  HRP Enzyme, 1  $\mu\text{L}$  Dye Reagent.

- Add 80  $\mu\text{L}$  of the WR to each well. Tap plate briefly to mix.

- Incubate at room temperature for 60 min. Use a plate reader to read  $\text{OD}_{570\text{nm}}$ .

##### Fluorimetric Procedure

Dilute the standards prepared in Colorimetric Procedure 1:10 in  $\text{dH}_2\text{O}$ .

Transfer 20  $\mu\text{L}$  standards and 20  $\mu\text{L}$  samples into separate wells of a *black 96-well plate*.

Add 80  $\mu\text{L}$  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  $\lambda_{\text{ex/em}} = 530/585$  nm.

#### CALCULATION

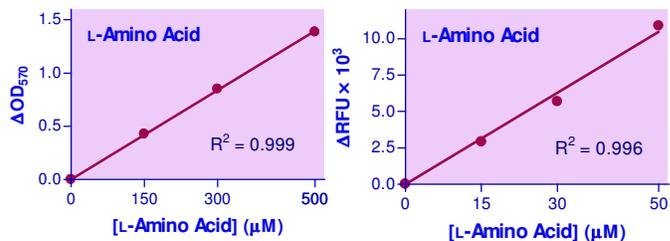
Subtract blank value (water, standard #4) from the standard values and plot the adjusted values against standard concentrations. Determine the slope and calculate the L-amino acid concentration of Sample as follows

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

where  $R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  are the OD or fluorescence values of the Sample and  $\text{H}_2\text{O}$  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

- 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.
- 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.
- Amelung, W., et al (2001) Determination of amino acid enantiomers in soil. *Soil Biology and Biochemistry*. 2001 Apr 1;15(4-5):553-562.

