L-Amino Acid Kit (BA0251)

Product Datasheet | SKU BA0251

Description

A quantitative colorimetric and fluorimetric assay for L-amino acids. An enzyme-catalysed oxidation of L-amino acids converts a dye into a coloured and fluorescent form measured at 570 nm (absorbance) or λex/em = 530/585 nm (fluorescence).

Specifications

Detection Method	Colorimetric (OD570nm) and fluorimetric (λex/em = 530/585 nm)
Detection Range	3.3 to 500 µM (colorimetric); 0.13 to 50 µM (fluorimetric)
Sample Type	Serum, culture media, tissue homogenates, cell lysates, urine and food samples
Species Reactivity	All
Assay Time	60 minutes
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	6 months after receipt
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Assay Buffer	12 mL	-20°C
LAA Enzyme	120 µL	-20°C
HRP Enzyme	120 µL	-20°C
Dye Reagent	120 µL	-20°C
Standard	1 mL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0251	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Sample preparation. Tissue or solid samples (e.g. food): homogenise 20-100 mg sample in 200-1000 µL dH2O, centrifuge at 10,000 × g for 15 min at 4°C and remove supernatant. Cell lysate: collect cells by centrifugation at 2,000 × g for 5 min at 4°C (use a rubber policeman for adherent cells), homogenise or sonicate in cold buffer containing 50 mM potassium phosphate (pH 7.5), centrifuge at 14,000 × g for 10 min at 4°C and remove supernatant. Liquid samples can be assayed directly; dilute serum and cell culture media 4-fold in dH2O, and for urine use an internal standard method.
2. Reagent preparation. Equilibrate all reagents to room temperature.
3. Colorimetric procedure - Standards: prepare 200 µL of 500 µM Premix by mixing 50 µL Standard (2 mM) and 150 µL dH2O. Dilute standards in 1.5-mL centrifuge tubes as in the standards table. Transfer 20 µL standards into separate wells of a clear flat-bottom 96-well plate.
4. Transfer 20 µL of each sample into separate wells.

5. Prepare enough Working Reagent (WR) for all wells by mixing, per well: 85 µL Assay Buffer, 1 µL LAA Enzyme, 1 µL HRP Enzyme and 1 µL Dye Reagent. Add 80 µL WR to each well. Tap the plate briefly to mix.
6. Incubate at room temperature for 60 min and read OD570nm.
7. Fluorimetric procedure: dilute the standards prepared above 1:10 in dH2O. Transfer 20 µL standards and 20 µL samples into separate wells of a black 96-well plate. Add 80 µL Working Reagent to each well and tap to mix. Incubate protected from light for 60 min at room temperature and read fluorescence at λex/em = 530/585 nm.

Standard Preparation (Colorimetric)

No	Premix + H2O	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

Calculation

Subtract the 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: [L-Amino Acid] = (R_SAMPLE – R_BLANK) / Slope (μM^{-1}) × n (µM), where R_SAMPLE and R_BLANK are the OD or fluorescence values of the sample and H2O blank respectively, and n is the sample dilution factor.

Notes

- Normal precautions for laboratory reagents should be exercised while using the reagents.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.