Technical Bulletin

L-Amino Acid Assay Kit

Catalogue number MAK490

Product Description

L-Amino acids are essential molecules defined by the nature of their sidechain. There are 20 amino acids that are commonly used by most organisms to produce proteins and other biologically relevant molecules. While amino acids can occur in either the D- or L-enantiomer, only the L-form is used by cells.

The L-Amino Acid Assay Kit 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 λ_{Ex}=530 nm/λ_{Em}=585 nm is directly proportional to the L-amino acid concentration in the sample.

The linear detection range of the kit is 3.3 – 500 µM for the colorimetric assay and 0.13 – 50 µM for the fluorometric assay. The kit is suitable for L-amino acid determination in serum, culture media, tissue homogenates, cell lysates, urine, food samples, etc.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer — 12 mL
  Catalogue Number MAK490A
- HRP Enzyme — 120 µL
  Catalogue Number MAK490B
- LAA Enzyme — 120 µL
  Catalogue Number MAK490C
- Dye Reagent — 120 µL
  Catalogue Number MAK490D
- Standard (2 mM) — 1 mL
  Catalogue Number MAK490E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor, pipette tips, etc.)
- Multiwell plate reader.
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.
- Dounce tissue grinder set.
  (Catalogue Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥ 14,000 × g
- Phosphate Buffered Saline (PBS)
  (Catalogue Number P3813 or equivalent)
- Potassium phosphate monobasic
  (Catalogue Number P0662 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Tissue or Solid Samples (for example, food)

1. Homogenize 20-100 mg of Sample in 200-1000 µL purified water.
2. Centrifuge at 10,000 × g for 15 minutes at 4 °C. Use the clear supernatant for the assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × g for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman or cell scraper.
3. Homogenize or sonicate cells in an appropriate volume of cold Assay Buffer containing 50 mM potassium phosphate (pH 7.5).
4. Centrifuge at 14,000 × g for 10 minutes at 4 °C. Use the clear supernatant for the assay.

Liquid Samples

Assay can be performed directly on liquid Samples. It is recommended to dilute serum and cell culture media Samples 4-fold in purified water prior to assay.

All Samples (Except Urine)

Transfer 20 µL of Sample to wells of a 96-well plate.

Urine Samples

1. Urine Samples must be diluted at least 5-fold with purified water.
2. Urine Samples require three separate reactions:
   a. Sample

Sample Plus Internal Standard

   b. Sample Blank
3. For the Internal Standard, prepare a 1250 µM Standard by mixing 125 µL of 2 mM Standard with 75 µL of purified water.
4. For the Sample Plus Internal Standard well, add 5 µL of the 1250 µM Internal Standard from Step 3 and 20 µL of Sample.
5. For the Sample and Sample Blank wells, add 5 µL of purified water and 20 µL Sample.

Colorimetric Standard Curve Preparation

1. Prepare a 500 µM Standard by mixing 50 µL of the 2 mM Standard and 150 µL of purified water.
2. Prepare Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of L-Amino Acid Colorimetric Standards

Well	500 µM Standard	Purified water	L-Amino Acid (µM)
1	100 µL	-	500
2	60 µL	40 µL	300
3	30 µL	70 µL	150
4	-	100 µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare Standards according to Colorimetric Standard Curve Preparation section.
2. Mix 10 µL of the Standards from Colorimetric Procedure with 90 µL of purified water according to Table 2.

Table 2.
Preparation of L-Amino Acid Fluorometric Standards

Well	Colorimetric Standard	Purified Water	L-Amino Acid (µM)
1	10 µL of 500 µM Std	90 µL	50
2	10 µL of 300 µM Std	90 µL	30
3	10 µL of 150 µM Std	90 µL	15
4	-	100µL	0

3. Mix well and transfer 20 µL of each Standard into separate wells of a black 96-well plate.

Working Reagent

1. Mix enough reagent for the number of assays to be performed. For each well, prepare 88 µL of Working Reagent according to Table 3.
   a. For urine Samples only, prepare 88 µL of Sample Blank Working Reagent for each Sample Blank well.

Table 3.

Preparation of Working Reagent

Reagent	Working Reagent	Sample Blank Working Reagent for Urine Samples
Assay Buffer	85 µL	86 µL
LAA Enzyme	1 µL	-
HRP Enzyme	1 µL	1 µL
Dye reagent	1 µL	1 µL

2. Transfer 80 µL of Working Reagent into each reaction well.

a. For urine samples, transfer 80 µL of Sample Blank Working Reagent to Sample Blank wells.

3. Tap plate to mix.

Measurement

1. Incubate at room temperature for 60 minutes. Protect plate from light for fluorometric assay.
2. Measure the optical density (OD) at 570 nm or fluorescence intensity (F) at λ_{Ex} = 530 nm/λ_{Em} = 585 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD or ΔF against standard concentrations and determine the slope of the Standard curve.
3. Calculate the L-Amino Acid concentration of Samples using the below equation:

$$\text{L-Amino Acid (µM)} = \frac{R_{Sample} - R_{Blank}}{\text{Slope } (\mu M^{-1})} \times DF$$

4. For urine Samples, follow Steps 1-2 above and then calculate the L-Amino Acid concentration of Samples using the below equation:

$$\text{Urine L-Amino Acid (µM)} = \frac{R_{Sample} - R_{Sample\ Blank}}{R_{Standard} - R_{Sample}} \times \frac{1250}{4} \times DF$$

where:

R_{Sample} = OD or fluorescence intensity (F) reading of Sample

R_{Blank} = OD or fluorescence intensity (F) reading of Blank

$R_{Sample\ Blank}$ = OD or fluorescence intensity (F) reading of Sample Blank (urine Samples only)

1250 = Concentration in µM of Internal Standard

4 = The volume of the Internal Standard is 4× lower than the Sample volume. In order to correct the calculation, the Internal Standard concentration is divided by 4.

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Note: The Standard is 2 mM L-leucine, therefore the results are in terms of µM of L-leucine.

Figure 1.
Typical Colorimetric L-Amino Acids Standard Curve

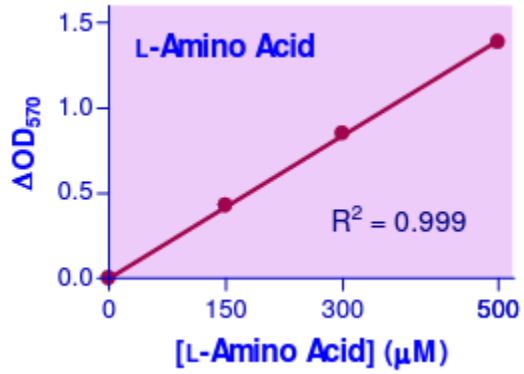
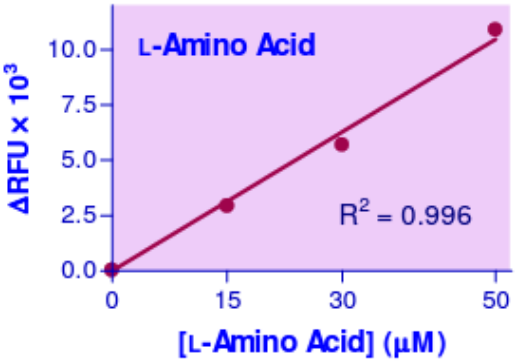


Figure 2.
Typical Fluorometric L-Amino Acids Standard Curve



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