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Product Information

L-Amino Acid Quantitation Kit

Catalog Number **MAK002**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

L-amino acids are essential molecules defined by the nature of their side-chain. There are 20 amino acids that are commonly used by most organisms for the production of proteins and other biologically relevant molecules. While amino acids can occur in either the D- or L-enantimer, only the L-form is used by cells.

In this assay, L-Amino Acid concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/ λ_{em} = 587 nm) product, proportional to the L-Amino Acids present. Glycine is not detectable by this assay.

Components

The kit is sufficient for 100 assays in 96 well plates.

L-Amino Acid Assay Buffer Catalog Number MAK002A	25 mL
L-Amino Acid Probe, in DMSO Catalog Number MAK002B	0.2 mL
L-Amino Acid Enzyme Mix Catalog Number MAK002D	1 vl
L-Amino Acid Standard, 4 nmole/µL Catalog Number MAK002E	0.3 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

L-Amino Acid Assay Buffer – Allow buffer to come to room temperature before use.

L-Amino Acid Probe – Thaw at room temperature to melt solution prior to use. Aliquot and store protected from light and moisture at –20 °C. Upon thawing, the L-Amino Acid Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the L-Amino Acid Probe Solution 5 to 10-fold with L-Amino Acid Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

L-Amino Acid Enzyme Mix – Reconstitute with 220 µL of L-Amino Acid Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

<u>L-Amino Acid Standards for Colorimetric Detection</u>
Add 0, 2, 4, 6, 8, and 10 µL of the L-Amino Acid Standard (4 nmole/µL) into a 96 well plate, generating 0 (blank), 8, 16, 24, 32, and 40 nmole/well standards. Add L-Amino Acid Assay Buffer to each well to bring the volume to 50 µL.
<u>Note:</u> The standard is an equimolar mixture of all proteinogenic amino acids with the exception of glycine.

L-Amino Acid Standards for Fluorometric Detection
Dilute 20 µL of the L-Amino Acid Standard (4 nmole/µL) with 180 µL of L-Amino Acid Assay Buffer to prepare a 0.4 nmole/µL standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the L-Amino Acid standard solution into a 96 well plate, generating 0 (blank), 0.8, 1.6, 2.4, 3.2, and 4.0 nmole/well standards. Add L -Amino Acid Assay Buffer to each well to bring the volume to 50 µL.
Note: The standard is an equimolar mixture of all proteinogenic amino acids with the exception of glycine.

Sample Preparation
Tissue (10 mg) or cells (2×10^6) should be rapidly homogenized in 4 volumes of cold L-Amino Acid Assay buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material.

Serum and other liquid samples can be directly added to the wells.

Bring samples to a final volume of 50 µL with L-Amino Acid Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Volume
L-Amino Acid Assay Buffer	46 µL
L-Amino Acid Probe	2 µL
L-Amino Acid Enzyme Mix	2 µL

2. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results
Calculations
The background for the assays is the value obtained for the 0 (blank) L-Amino Acid Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate L-Amino Acid standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Concentration of L -Amino Acids

$$S_a/S_v = C$$

S_a = Amount of L-Amino Acids in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added into the wells
C = Concentration of L-Amino Acids in sample

Sample Calculation
Amount of L-Amino Acid (S_a) = 5.84 nmole (from standard curve)
Sample volume (S_v) = 50 µL

Concentration of L-Amino Acids in sample

5.84 nmole/50 µL = 0.1168 nmole/µL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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