ab65347

L-Amino Acid Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of L-Amino Acid levels in various samples.

This product is for research use only and is not intended for diagnostic use.

Version 3 Last Updated 29 January 2019

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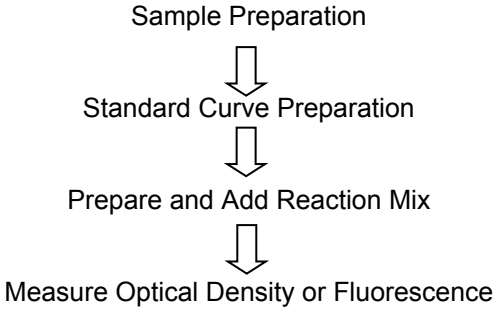
1. Overview

L-Amino acids are the most essential elements in biology. Accurately quantitating L-amino acids in body fluids or purified samples may provide valuable information for diagnostic or basic research studies. Abcam's L-Amino Acid Assay Kit provides a convenient means for directly detecting L-amino acid in biological samples.

There is no requirement for sample pre-treatment or purification when using this kit. The L-amino acid(s) level can be quantified using fluorometric (at Ex/Em = 535/587 nm) or colorimetric (at λ = 570 nm) methods in 96-well plates.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
L-Amino Acid Assay Buffer	25 mL
L-Amino Acid Assay Probe	0.2 mL
L-Amino Acid Enzyme Mix	1 vial
L-Amino Acid Standard (4 nmol/µl)	0.3 mL

* Store the kit at -20°C. Read the entire protocol before performing the assay.

The L-Amino Acid Standard is a mixture of all amino acids at equal molar ratio. Glycine cannot be detected by this kit.

PROBE: Ready to use as supplied. Warm to room temperature to thaw the DMSO solution before use. Store at –20°C, protect from light. Use within two months.

ENZYME MIX: Dissolve in 220 µl L-Amino Acid Assay Buffer. Pipette up and down to complete dissolve the content. Store at -20°C. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Prepare test samples in 50 µl/well with L-Amino Acid Assay Buffer in the 96-well plate.

We suggest using several doses of your sample to ensure the readings are within the standard curve range.

2. Standard Curve Preparation:

a. For the colorimetric assay:

Add 0, 2, 4, 6, 8, 10 µl L-Amino Acid Standard into each well individually of a 96-well plate to generate 0, 8, 16, 24, 32, 40 nmol/well of L-Amino Acid Standard. Adjust volume to 50 µl/well with L-Amino Acid Assay Buffer.

b. For the fluorometric assay:

Dilute the L-Amino Acid to 0.4 nmol/µl by adding 10 µl of the L-Amino Acid to 90 µl of L-Amino Acid Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually to generate 0, 0.8, 1.6, 2.4, 3.2, 4.0 nmol/well of the L-Amino Acid Standard. Adjust volume to 50 µl/well with L-Amino Acid Assay Buffer.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

L-Amino Acid Assay Buffer	46 µl
L-Amino Acid Probe	2 µl
L-Amino Acid Enzyme Mix	2 µl

The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease the background reading/increase detection sensitivity significantly.

Mix well. Add 50 µl of the Reaction Mix to each well containing the L-Amino Acid standard or test samples.

4. Incubate the reaction for 30 min at 37°C, protect from light.

5. Measure OD_{570nm} for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a micro-plate reader.

5. Data Analysis

Correct background by subtracting the value derived from the zero L-Amino Acid control from all sample readings. The background reading can be significant and must be subtracted from sample readings.

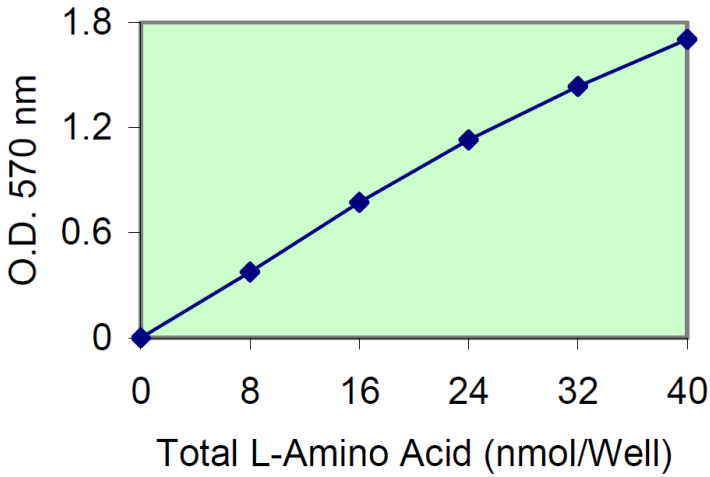
Apply the sample readings to the L-amino acid standard curve to obtain the total amino acid amount.

L-Amino Acid Concentration = A / Sv (nmol/µl or mM)

Where:

A is the L-Amino acid amount (nmol) from the standard curve based on Absorbance OD_{570nm} or fluorescence of your samples.

Sv is the Sample volume (µl) you added into the sample wells.



L-Amino Acid Standard Curve. Different doses of L-Amino Acids were measured according to the kit procedure.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended sample types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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