BioVision

For research use only

L-Amino Acid Quantitation Colorimetric/Fluorometric Kit

(Catalog #K639-100; 100 assays; Store kit at –20°C)

I. Introduction:

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. BioVision's L-Amino Acid Assay Kit provides a convenient means for directly detecting L-amino acids in biological samples. There is no requirement for sample pretreatment or purification when using this kit. The L-amino acid levels can be quantified using fluorometric (at Ex/Em = 535/587 nm) or colorimetric (at λ = 570 nm) methods in 96-well plates.

II. Kit Contents:

Components	K639-100	Cap Code	Parts Number
L-Amino Acid Assay Buffer	25 ml	WM	K639-100-1
L-Amino Acid Probe	0.2 ml	Red	K639-100-2A
L-Amino Acid Enzyme Mix	1 Vial	Green	K639-100-4
L-Amino Acid Standard (4nmol/µl)*	300µl	Yellow	K639-100-5

*Mixture of all amino acids at equal molar ratio. Glycine is not detectable in this assay.

III. Reagent Preparation and Storage Conditions:

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.

IV. Assay Protocol:

1. **Standard Curve Preparations:** For the colorimetric assay, add 0, 2, 4, 6, 8, 10 µl L-Amino Acid Standard into a series of wells 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.

   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.

2. **Sample Preparations:** Prepare test samples ina final volume of 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.

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

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

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

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

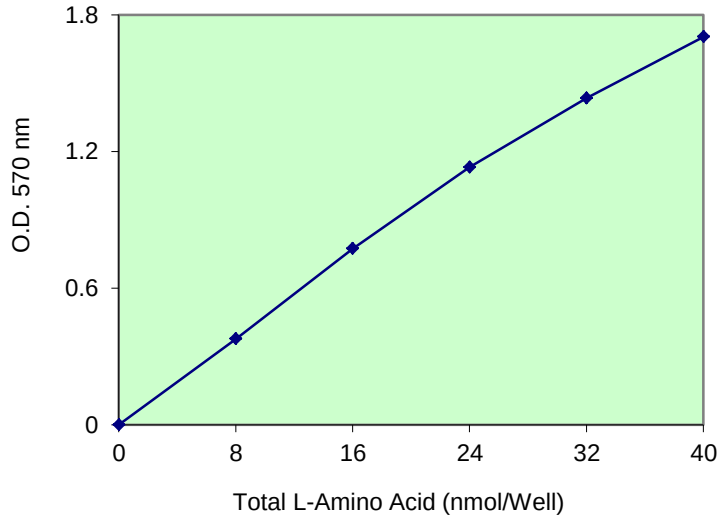
6. Measure the OD at 570nm for the colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a micro-plate reader.

7. Correct background by subtracting the value of the 0 L-amino acid control from all samples (The background reading can be significant and must be subtracted from sample readings). Then 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: L-Amino acid amount (nmol) from the standard curve based on Absorbance OD 570 or fluorescence of your samples.

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



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

V. Related Products:

Cholesterol/Cholesteryl Esters Quantitation Kit
HDL, LDL/VLDL cholesterol assay Kit
Glucose Assay Kit
Lactate Assay Kit
Glutathione Assay Kit
NAD/NADH Assay Kit
LDH-cytotoxicity Assay Kit
Uric Acid Assay Kit
Apoptosis Detection Kits

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Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		