

L-Amino Acid Quantitation Colorimetric/Fluorometric Kit (#BN00863)

(Catalog #BN00863; 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. Assay Genie'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 $\lambda = 570$ nm) methods in 96-well plates.

II. Kit Contents:

Components	BN00863	Cap Code	Parts Number
L-Amino Acid Assay Buffer	25 ml	WM	BN00863-1
L-Amino Acid Probe	0.2 ml	Red	BN00863-2
L-Amino Acid Enzyme Mix	1 Vial	Green	BN00863-3
L-Amino Acid Standard (4nmol/ μ l)*	300 μ l	Yellow	BN00863-4

*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 μ l 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:

- Standard Curve Preparations:** For the colorimetric assay, add 0, 2, 4, 6, 8, 10 μ l 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 μ l/well with L-Amino Acid Assay Buffer.

For the fluorometric assay, dilute the L-Amino Acid to 0.4 nmol/ μ l by adding 10 μ l of the L-Amino Acid to 90 μ l of L-Amino Acid Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 μ l 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 μ l/well with L-Amino Acid Assay Buffer.

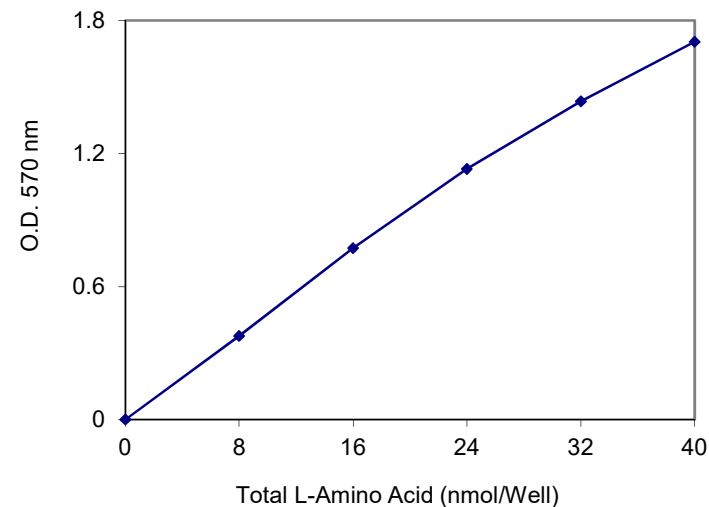
- Sample Preparations:** Prepare test samples in a final volume of 50 μ l/well with L-Amino Acid Assay Buffer in the 96-well plate. We suggest using several doses of your sample to ensure the readings are within the standard curve range.
- Reaction Mix Preparation:** Mix enough reagents for the number of assays performed: For each well, prepare a total 50 μ l Reaction Mix containing:
 - 46 μ l L-Amino Acid Assay Buffer
 - 2 μ l L-Amino Acid Probe
 - 2 μ l L-Amino Acid Enzyme Mix,
- Add 50 μ l of the Reaction Mix to each well containing the L-Amino Acid standard or test samples.
- Incubate the reaction for 30 min at 37°C, protect from light.

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

$$\text{L-Amino Acid Concentration} = A/Sv \text{ (nmol}/\mu\text{l or mM)}$$

Where A: L-Amino acid amount (nmol) from the standard curve based on Absorbance OD 570 or fluorescence of your samples.

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



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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • 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
<p>Note# The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.</p>		