

# L-Amino Acid Quantitation Kit

(Catalog #K639-100; 100 assays; Store kit at  $-20^{\circ}\text{C}$ )

## 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 acid in biological samples. There is no requirement for sample pretreatment 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  $\lambda = 570$  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 Assay Probe	1 Vial	Red	K639-100-2
DMSO (Dried)	500 $\mu\text{l}$	Brown	K639-100-3
L-Amino Acid Enzyme Mix	1 Vial	Green	K639-100-4
L-Amino Acid Standard (4nmol/ $\mu\text{l}$ )*	300 $\mu\text{l}$	Yellow	K639-100-5

\*Mixture of all amino acids at equal molar ratio. Glycine can't be detected by this kit.

## III. Reagent Preparation and Storage Conditions:

**Probe:** Dissolve the probe with 220  $\mu\text{l}$  of dried DMSO (provided) before use. Mix, store at  $-20^{\circ}\text{C}$ , protect from light and moisture. Use within two months.

**Enzyme Mix:** Dissolve in 220  $\mu\text{l}$  L-Amino Acid Assay Buffer. Pipette up and down to complete dissolve the content. Store at  $-20^{\circ}\text{C}$ . Use within two months.

## IV. Assay Protocol:

- Standard Curve Preparations:** For colorimetric assay, add 0, 2, 4, 6, 8, 10  $\mu\text{l}$  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  $\mu\text{l}$ /well with L-Amino Acid Assay Buffer.

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

- Sample Preparations:** Prepare test samples in 50  $\mu\text{l}$ /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  $\mu\text{l}$  Reaction Mix containing:

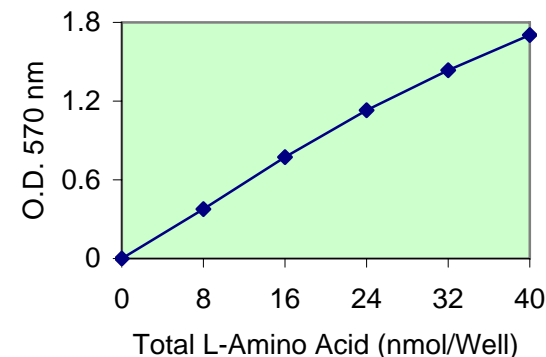
46  $\mu\text{l}$  L-Amino Acid Assay Buffer  
 2  $\mu\text{l}$  L-Amino Acid Probe  
 2  $\mu\text{l}$  L-Amino Acid Enzyme Mix,  
 Mix well.

- Add 50  $\mu\text{l}$  of the Reaction Mix to each well containing the L-Amino Acid standard or test samples.
- Incubate the reaction for 30 minutes at  $37^{\circ}\text{C}$ , protect from light.
- Measure O.D. 570nm for 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.

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

Where A: L-Amino acid amount (nmol) from the standard curve based on Absorbance OD570 or fluorescence of your samples.

Sv: Sample volume ( $\mu\text{l}$ ) you added into the sample wells.



**L-Amino Acid Standard Curve.** Different doses 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