L-Arginine Microplate Assay Kit

User Manual

Catalog # CAK1277

(Version 1.1A)

Detection and Quantification of L-Arginine Content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids media Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

L-Arginine is a proteogenic, semi-essential amino acid: healthy humans can synthesize L-Arginine using L-Glutamine as a building block; however, premature infants are unable to produce Arg and additional supplementation is required for proper growth and development. Arginine plays pivotal roles in biochemical pathways such as the urea cycle and the biosynthesis of nitric oxide. Arginine and Ammonia concentrations are elevated in patients having a mutation in their ARG1 genes. The mutation causes lower arginase activities - a condition that is known as Argininemia. Arginine has also been advertised as a supplement due to its role in the synthesis of nitric oxide, which helps in vasodilation processes.

L-Arginine Microplate Assay Kit is designed to measure L-Arginine directly in biological samples without any pretreatment. In this enzyme-based assay, L-arginine is converted into a series of intermediates, which will further react with a probe producing a stable colorimetric signal, measured at 525nm, is directly proportional to the L-Arginine concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Enzyme	Powder x 1	-20 °C
Reaction Buffer	5 ml x 1	4 °C
Stop Solution	10 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use.

Dye Reagent: add 5 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, mix, the concentration will be 10 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 525 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For urine, serum or other biological fluids samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Reaction Buffer	30 µl	30 µl	30 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Enzyme	10 µl	10 µl	10 µl
Shake and mix, put it into the oven, 37 °C for 20 minutes.			
Stop Solution	100 µl	100 µl	100 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, put the plate into the convection oven, 90 °C for 20 minutes. When cold, record absorbance measured at 525 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{L-Arginine (μmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{L-Arginine (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{L-Arginine (μmol/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{L-Arginine (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the concentration of Standard, 10 mmol/L = 10 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

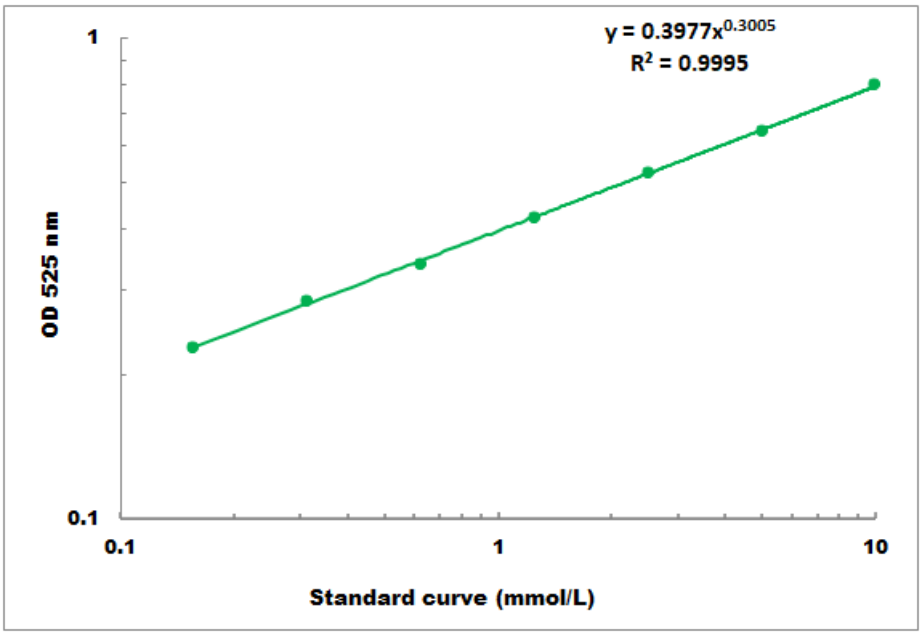
$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES