L-Arginine

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1277

(Version 1.2A)

Detection and Quantification of L-ArginineContentin Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids mediaSamples.

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-ArginineColorimetric 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	Powderx 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 mldistilled waterto generate 10 mmol/L of standardtop solution,store at 4 °C for 1 monthor -20°C for 6 months after reconstitution.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve.The concentration of standard curve could be10/5/2.5/1.25/0.625/0.312/0.156 mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Bufferto dissolve before use.Store at 4 °C for 1 week or -20C for 6 monthsafter reconstitution.

Dye Reagent: Add 5 ml distilled water to dissolve before use.Store at -20°C for 1-2 monthsafter reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For urine, serum orother biological fluidssamples

Detect directly.

VI. 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 20minutes.			
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 20minutes. When cold, record absorbance measured at 525 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times C_{Protein}\times V_{Sample}}(\mu mol/mg)$$

2.2According to the quantity of cells or bacteria

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times N\times(V_{Sample}/V_{Assay})}(\mu mol/10^{4})$$

2.3 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.4According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

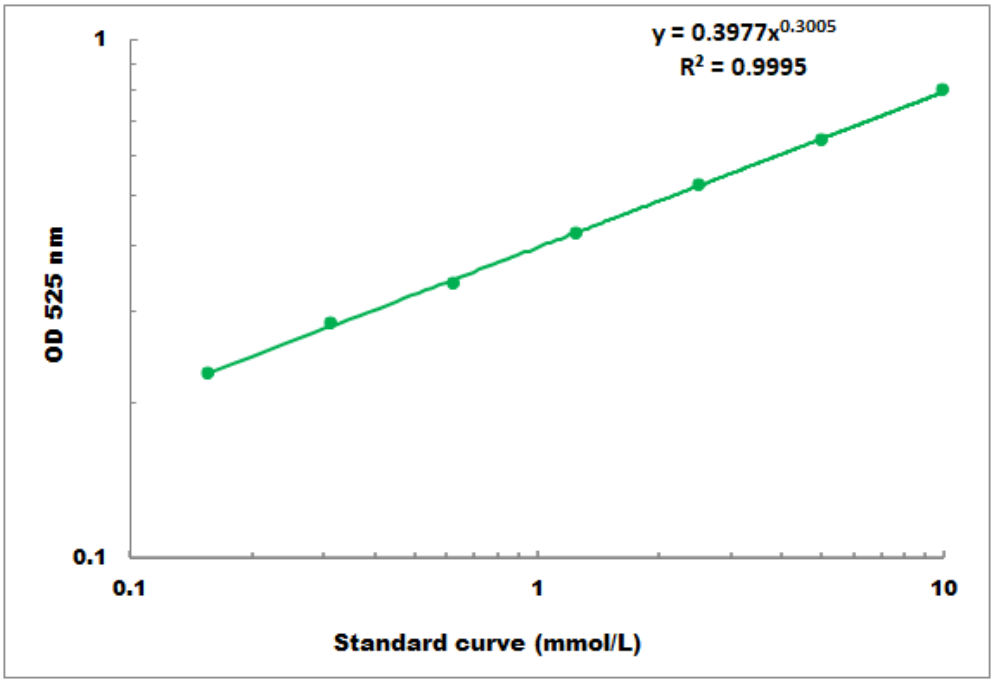
$C_{Protein}$: the sample protein concentration, mg/ml

W: the weight of sample, g

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

VIII. TYPICAL DATA

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



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