



Arginase Microplate Assay Kit

User Manual

Catalog # CAK1118

(Version 1.2A)

Detection and Quantification of Arginase Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

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I. INTRODUCTION

Arginase (EC 3.5.3.1) is present in mammals and plants. In humans, arginase is expressed predominantly in the liver, and to lesser degrees in breast, kidney, testes, salivary glands, epidermis and erythrocytes. Arginase catalyzes the conversion of arginine to ornithine and urea, completing the last step in the urea cycle. Arginase activity is a key diagnostic indicator. Increased levels of arginase activity in blood have been associated with liver damage. Hyperargininemia due to arginase deficiency is an inherited autosomal recessive disease.

Arginase Microplate Assay Kit provides a sensitive and convenient method for arginase activity determination. The method utilizes a chromogen that forms a colored complex specifically with urea produced in the arginase reaction. The intensity of the color, measured at 525nm, is directly proportional to the arginase activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	4 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
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
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Note:

Substrate: add 4 ml Substrate Diluent 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; then add 0.03 ml into 0.97 ml distilled water. The concentration will be 3 mmol/L.

Positive Control: add 0.1 ml Assay Buffer to dissolve before use.

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. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all the reagents to 37°C before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Substrate	40 µl	40 µl	40 µl	40 µl	40 µl
Sample	10 µl	--	--	--	--
Assay Buffer	--	10 µl	--	--	--
Positive Control	--	--	--	--	10 µl
Standard	--	--	10 µl	--	--
Distilled water	--	--	--	10 µl	--
Shake and mix, put it into the oven, 37 °C for 10 minutes.					
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Dye Reagent	50 µl	50 µl	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) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.
- 3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: 1 unit of arginase activity is defined as the enzyme causes the hydrolysis of 1 μmol arginine per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{Arginase (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (C_{\text{Protein}} \times V_{\text{Sample}}) / T \\ &= 0.3 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{Arginase (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times W / V_{\text{Assay}}) / T \\ &= 0.3 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{Arginase (U}/10^4) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times N / V_{\text{Assay}}) / T \\ &= 0.3 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

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

C_{Standard} : the concentration of Standard, 3 mmol/L = 3 $\mu\text{mol}/\text{ml}$;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

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

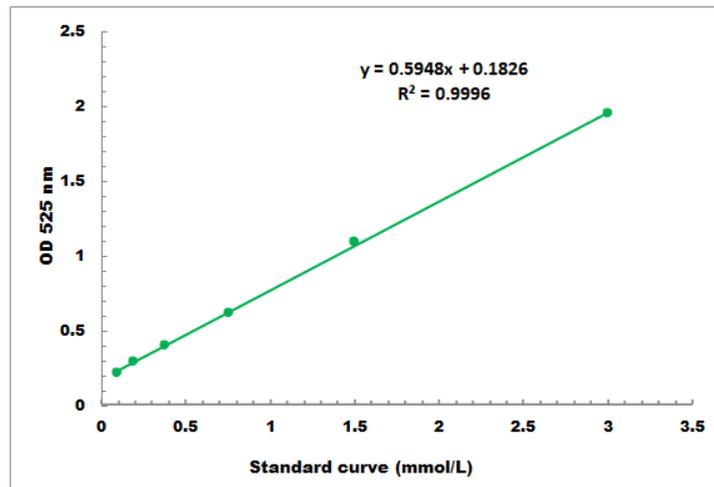
V_{Sample} : the volume of sample, 0.01 ml;

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

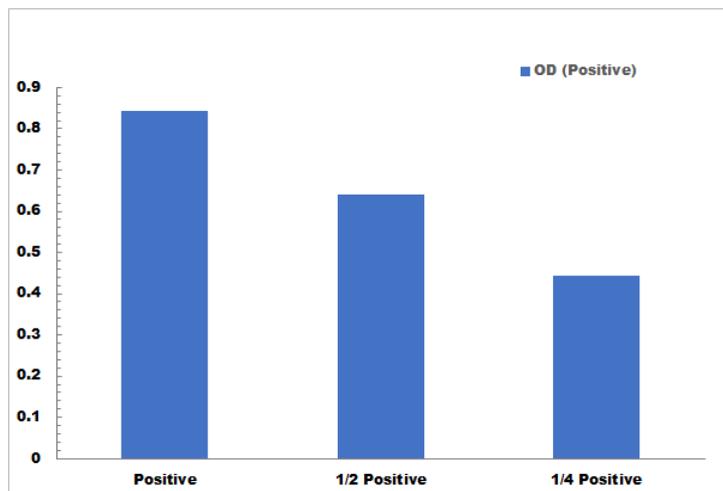
T: the reaction time, 10 minutes.

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 - 3 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

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

IX. NOTES