



Arginase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1118

(Version 1.3A)

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 Activity Colorimetric 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	
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. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 100 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 3 mmol/L standard top solution by adding 30 μ l stock solution into 970 μ l distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 3/1.5/0.75/0.375/0.188/0.094/0.047 mmol/L.

Substrate: Add 4 ml Substrate Diluent to dissolve before use. Store at 4 °C for 1-2 weeks or -20°C for 4-5 months after reconstitution.

Dye Reagent: Add 5 ml distilled water to dissolve before use. Keep in dark for immediate use or store at -20°C for 1-2 weeks after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.1 ml Assay Buffer to dissolve before use. Store at -80°C for 1 month after reconstitution.

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

V. 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.

VI. ASSAY PROCEDURE

Warm all thereagents to 37°C before use.

Add following reagents intothe 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 10minutes.					
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 20minutes. When cold, record absorbance measured at 525 nm.					

Note:

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

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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

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 slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

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

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein} : the protein concentration of sample, mg/mL

W: the weight of total sample, g

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

C_{Standard} : the concentration of standard, mmol/L = $\mu\text{mol/mL}$

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

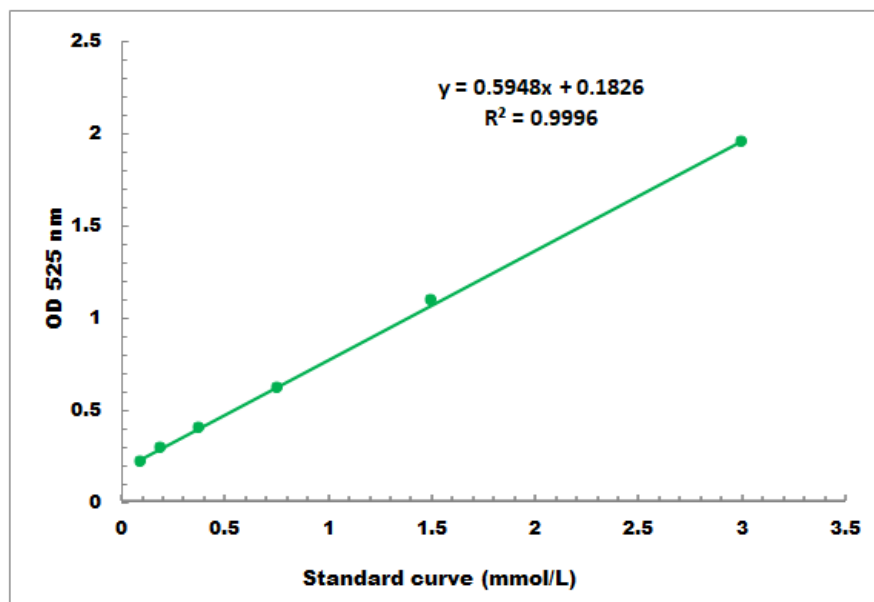
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

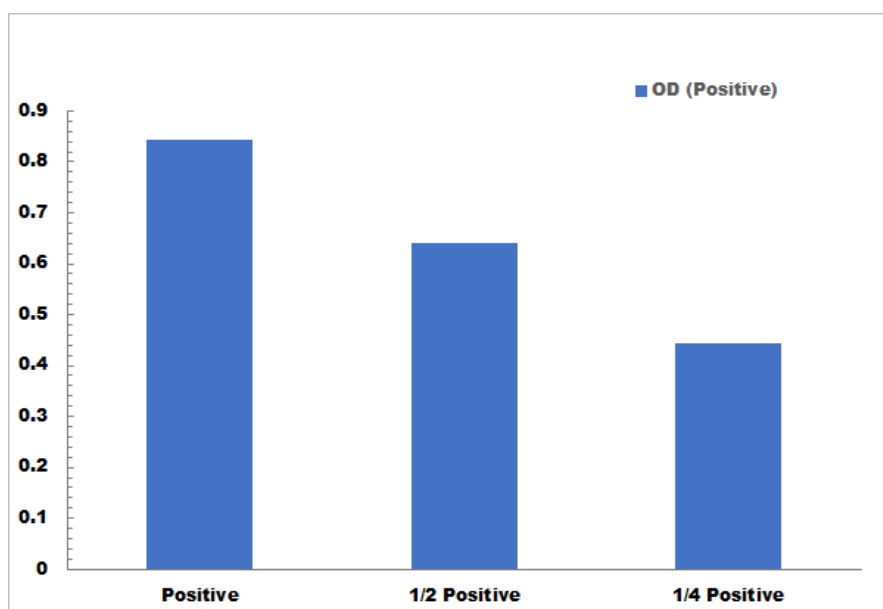
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.1mmol/L -3mmol/L



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