Arginase Assay Kit

RG83431 Arginase Assay Kit can be used to measure Arginase in Serum, plasma, tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83431

Package: 96 wells

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INTRODUCTION

Arginase (E.C.3.5.3.1) is a mammalian enzyme which catalyzes the conversion of arginine to ornithine and urea. Arginase is considered as an enzyme responsible for the cyclic nature of urea cycle, since only the organisms containing arginase are able to carry out the complete urea cycle. Two distinct isoforms of mammalian arginase have been identified that are encoded by two separate genes. Type I arginase (arginase I) is located in the cytosol and is mainly expressed in liver. Type II arginase is located in the mitochondrial matrix and is expressed in extra-hepatic tissues.

PRINCIPLE OF THE ASSAY

The Arginase Assay Kit utilizes a chromogen witch can forms colored complex specifically with urea produced in the arginase reaction. The enzyme catalyzed reaction products can be measured at a colorimetric readout at 525 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4°C
Positive Control	1 vial (lyophilized)	-20°C
Assay Buffer	4 X 30 ml (ready to use)	4°C
Substrate Diluent	4 ml	4°C
Reaction Dye	1 vial (lyophilized)	4°C
Substrate	1 vial (lyophilized)	4°C
Stop Solution	10 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 525 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Convection oven (37°C and 90°C)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Positive Control store at -20°C, all other component store at 4°C.
- Reaction Dye should be store at 4°C and protect from light.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell or bacteria lysate- Collect cell or bacteria in a centrifuge tube, wash cells 1-2X by PBS. Discard the supernatant after centrifugation, add 1 ml of Assay buffer pre 5×10^6 cell or bacteria in the tube. And then sonicate samples (set with power 20%, sonicate for 3 sec. and interval for 10 sec., repeat 30 times). Centrifuge samples 8000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay.

Tissue lysate- Weigh out 0.1 g of tissue, homogenize with 1 ml Assay buffer on ice. Centrifuge samples 8000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Standard:** Add **1 ml** of **distilled water** to dissolve before use (to yield 100µmol/ml as stock); then add 0.03 ml into 0.97 ml distilled water. The concentration will be 3 µmol/ml. Perform 2-fold serial dilution of the top standards to make the standard curve.
- **Reaction Dye**: Reconstitute the Substrate with **5 ml** of **distilled water**. Allow the Reaction Dye keep on bench for few minutes. Make sure the Reaction Dye is dissolved completely and mixed thoroughly before use. Keep the reconstituted the Substrate on ice before use.
- **Substrate:** Reconstitute the Substrate with **9 ml** of **Substrate Diluent**. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use. Keep the reconstituted the Substrate on ice before use. Keep the reconstituted the Substrate on ice before use.

- **Positive Control:** Dilute the Positive Control with **100 µl** of **Assay Buffer.** Make sure the Positive Control is mixed thoroughly before use. Keep the Positive Control on ice before use.
- **Sample**: If the measuring absorbance of samples is higher than the standard, dilute the samples with **1X Assay buffer** before assay and assay again. For the calculation of the activity this dilution factor has to be taken into account.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **40 µl Substrate** to All wells.
2. Sample wells: Add **10 µl** per **samples** into each microplate.
3. Control wells: Add **10 µl** of **Assay Buffer** into another microplate.
4. Positive Control wells: Add **10 µl** of **Positive Control** into microplate.
5. Standard wells: Add **10 µl** of **Standard** into microplate.
6. Mix well and incubate all Samples, Control and Positive Control tubes at **37°C** oven for **10 min**.
7. Add **100 µl** of **Stop Solution** per well into all wells.
8. Add **50 µl** of **Reaction Dye** per well into all wells.
9. Mix well. Incubate at **90°C** oven for **20 min**.
10. Read the OD with a microplate reader at **525 nm**.

Reagent	Sample	Control	Positive Control	Standard	Blank
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
Mix well. Incubate all Sample tubes at **37°C** oven for **10 min**.					
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Reaction Dye	50 µl	50 µl	50 µl	50 µl	50 µl
Mix well. Incubate at **90°C** oven for **20 min**.					
Read the OD with a microplate reader at **525 nm**.					

CALCULATION OF RESULTS

1. Unit Definition: One unit activity is defined as the enzyme that hydrolysis of one µmol arginine per minute.

2. Calculate the average absorbance values for each set of samples, standard, positive control, control and blank.

3. Calculation:

A. Definition:

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

$C_{Standard}$: the standard concentration, 3 µmol/ml;

W: the weight of sample, g;

V_{Sample}: the volume of reaction sample, 10 µl = 0.01 ml;

$V_{standard}$: the volume of standard sample, 10 µl = 0.01 ml;

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

T: the reaction time, 10 minutes.

B. Formula:

a). According to the protein concentration of sample

Arginase activity (U/mg) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample}-OD_{Control})] / [(OD_{Standard}- OD_{Blank}) \times C_{Protein} \times V_{Sample} \times T]$

$=0.3 \times (OD_{Sample}-OD_{Control}) / [(OD_{Standard}- OD_{Blank}) \times C_{Protein}]$

b). According to the weight of sample

Arginase activity (U/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample}-OD_{Control})] / [(OD_{Standard}- OD_{Blank}) \times W \times (V_{Sample} / V_{total}) \times T]$

$= 0.3 \times (OD_{Sample}-OD_{Control}) / [(OD_{Standard}- OD_{Blank}) \times W]$

c). According to the quantity of cells or bacteria

Arginase activity ($U/10^4$) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample}-OD_{Control})] / [(OD_{Standard}- OD_{Blank}) \times N \times (V_{Sample} / V_{total}) \times T]$

$=0.3 \times (OD_{Sample}-OD_{Control}) / [(OD_{Standard}- OD_{Blank}) \times N]$

4. Detection range:

The detection range is from 100 μmol/ml – 3000 μmol/ml.

5. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serial diluted standards are not necessary for this kit.