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Arginase Activity Colorimetric Assay Kit

rev 01/20

(Catalog # K755-100; 100 assays; Store at -20°C)

I. Introduction:

Arginase (EC 3.5.3.1) is the final enzyme of the Urea Cycle. It converts L-arginine into urea and L-ornithine and plays an important role in removing ammonium ion from the body. Arginase has two isoforms: Arginase I and Arginase II. Arginase I is mainly present in the liver and plays an important part in the urea cycle whereas Arginase II is present in kidney and other tissues and regulates Arginine/Ornithine concentration. Arginase deficiency can lead to severe symptoms including neurological impairment, dementia and hyperammonemia. Analysis of Arginase activity is fundamental to the study of the urea metabolic pathway. BioVision's Arginase Activity Assay kit is simple, sensitive and rapid. In this assay, Arginase reacts with arginine & undergoes a series of reactions to form an intermediate that reacts stoichiometrically with OxiRed™ Probe to generate the colored product (OD 570 nm). The kit can detect Arginase activity less than 1 mU/well.

L-Arginine $\xrightarrow{\text{Arginase}}$ Intermediate $\xrightarrow{\text{Probe}}$ Color Detection (OD 570 nm)

II. Application:

- Measurement of Arginase activity in various tissues/cells
- Analysis of urea cycle disorders

III. Sample Type:

- Animal tissues: liver, heart, kidney, etc.
- Cell culture: Adherent and suspension cells

IV. Kit Contents:

Components	K755-100	Cap Code	Part Number
Arginase Assay Buffer	25 ml	WM	K755-100-1
OxiRed™ Probe (in DMSO)	200 µl	Red	K755-100-2A
Arginase Substrate (Lyophilized)	1 vial	White	K755-100-3
Arginase Enzyme Mix (Lyophilized)	1 vial	Green	K755-100-4
Arginase Developer (Lyophilized)	1 vial	Orange	K755-100-5
Arginase Converter Enzyme (Lyophilized)	1 vial	Blue	K755-100-6
H_2O_2 Standard (0.88 M)	100 µl	Yellow	K755-100-7
Arginase Positive Control (Lyophilized)	1 vial	Purple	K755-100-8

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Arginase Assay Buffer to room temperature (RT) before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:

- **OxiRed™ Probe:** Ready to use as supplied. Aliquot and store at -20°C. Warm to RT before use. Use within two months.
- **Arginase Substrate:** Reconstitute with 220 µl dH_2O. Store at -20°C. Keep on ice while in use. Use within two months.
- **Arginase Enzyme Mix, Developer and Converter Enzyme:** Reconstitute with 220 µl Arginase Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.
- **Arginase Positive Control:** Reconstitute with 100 µl Arginase Assay Buffer and dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

VIII. Arginase Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold Arginase Assay Buffer on ice. Centrifuge at 10,000 X g for 5 min. Collect the supernatant. Add 1-40 µl sample(s) per well. Adjust the final volume to 40 µl with Arginase Assay Buffer. For samples with high background, prepare parallel sample well(s) as the background control to correct for interference from the urea in the sample. Add 0.2-2 µl of Arginase Positive Control into the desired well(s) & adjust the final volume to 40 µl with Arginase Assay Buffer.

   Note:

   a. High urea content in samples will interfere with the assay. To remove urea from samples, we suggest using a 10 kDa spin column (BioVision Cat.# 1997). In brief, add 50-200 µl of sample into a spin column. Centrifuge at 15,000 X g for 2 min. Replenish the lost liquid and repeat 2 times, discarding the filtrate, and bringing the sample (retentate) to its original volume with Arginase Assay Buffer.

   b. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

2. **Substrate Mix:** Make enough substrate mix for samples, sample background control(s) and Positive Control. For each well, prepare 10 µl substrate mix containing:

	Substrate Mix	*Background Control Mix
Arginase Assay Buffer	8 µl	10 µl
Arginase Substrate	2 µl	---

Mix. Add 10 µl Substrate Mix to samples and Positive Control. Mix well & Incubate for 20 min. at 37°C.

* For samples having high background, add 10 µl Background Control Mix to sample background control well(s). Mix well & incubate for 20 min. at 37°C.

3. **Standard Curve Preparation:** Dilute H_2O_2 Standard to 10 mM by adding 4 µl of 0.88 M H_2O_2 Standard to 348 µl dH_2O. Dilute further to 1 mM by adding 100 µl of 10 mM H_2O_2 Standard to 900 µl dH_2O. Add 0, 2, 4, 6, 8 and 10 µl of 1 mM H_2O_2 Standard into a series of wells in 96-well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of H_2O_2 Standard. Adjust the volume to 50 µl/well with dH_2O.

Note:

Diluted H_2O_2 Standard is unstable. Discard diluted Standard after use.

4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
Arginase Assay Buffer	42 µl
Arginase Enzyme Mix	2 µl
Arginase Developer	2 µl
Arginase Converter Enzyme	2 µl
OxiRed™ Probe	2 µl

Add 50 µl of Reaction Mix to each well containing Standards, Positive Control, test samples, & sample background control(s). Mix well.

5. **Measurement:** Measure absorbance (OD 570 nm) immediately in kinetic mode for 10-30 min. at 37ºC.

Note: Incubation time depends on the Arginase Activity in the samples. We recommend measuring the OD in a kinetic mode, and choosing two time points (T_1 & T_2) in the linear range to calculate the Arginase Activity of the samples. The H_2O_2 Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).

6. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the H_2O_2 Standard Curve. Correct sample reading by subtracting the value derived from the background control reading from sample reading. Calculate the Arginase Activity of the test samples: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the H_2O_2 Standard Curve to get B nmol of H_2O_2 generated by Arginase during the reaction time ($\Delta T = T_2 - T_1$).

Sample Arginase Activity = B/(ΔT X V) x Dilution Factor = nmol/min/µl = mU/µl or U/ml

Where: **B** is the H_2O_2 amount from Standard Curve (nmol).
Δ**T** is the reaction time (min.).
V is the sample volume added into the reaction well (µl).

Unit Definition: One unit of Arginase is the amount of enzyme that will generate 1.0 µmol of H_2O_2 per min. at pH 8 at 37ºC.

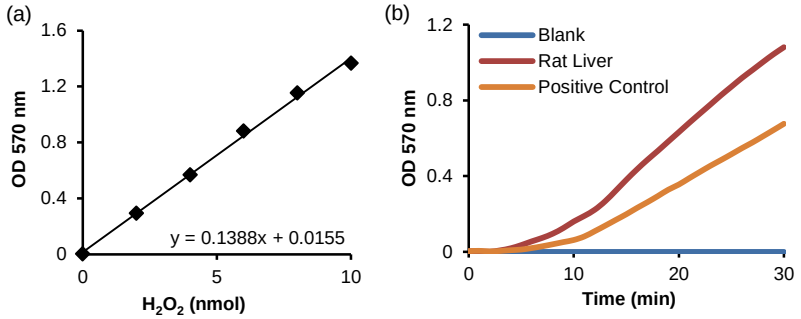

Figure: (a) H_2O_2 Standard Curve. (b) Arginase activity in rat liver lysate (3 µg) & Positive Control (2 µl). Assays were performed following the kit protocol.

IX. Related Products:

Urea Colorimetric Assay Kit (K375)
Fumarate Colorimetric Assay Kit (K633)
Ammonia Colorimetric Assay Kit II (K470)
10 kDa Spin Column (1997)
Urea Colorimetric Assay Kit II (K376)
Ammonia Colorimetric Assay Kit (K370)
Aspartate Colorimetric/Fluorometric Assay kit (K552)

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