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Arginase Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate Reader

Catalog Number: AK0776-100T-48S

Size: 100T/48S

Components: Please carefully check the volume of the reagent and the volume in the bottle before use.

Reagent name	Size	Preservation condition
Extract solution I	Liquid 60mL×1	2-8℃ storage
Extract solution II	Liquid 0.6mL×1	-20℃ storage
Reagent I	Powder ×1	2-8℃ storage
Reagent II	Liquid 4 mL×1	2-8℃ storage
Reagent III	Liquid 10 mL×1	2-8℃ storage
Reagent IV	Liquid 13 mL ×1	2-8℃ storage
Reagent V	Liquid 5.5 mL ×1	2-8℃ storage
Standard	Liquid 1 mL ×1	2-8℃ storage

Extract solution： According to the number of samples，prepare in the ratio of Extract I: Extract II = 990μL: 10μL (1T).It is forbidden to add all of Extract II to Extract I at once.

Reagent I: Dissolve well with 3.2mL of reagent II before use. Unused reagents can be stored at 2-8°C for up to 4 weeks. Do not store at -20°C.

Standard: 1 mol/L (1000 μmol/mL) urea standard solution.

(Extract solution II and reagent V are volatile reagents, unscrew the cap and seal it in time after use.)

Product Description:

Arginase, also known as L-arginine urea hydrolase or L-arginine amidinyl hydrolase, is a manganese metalloenzyme. Arginase is found in bacteria, yeast, plants, invertebrates and vertebrates, and is thought to have first appeared in bacteria. The main function of arginase in microorganisms is probably to participate in maintaining the dynamic balance of L-arginine and in regulating a variety of important metabolic processes.

Arginase catabolic L-arginine into L-ornithine and urea, urea reacts with α-isonitrosopropiophenone to produce a derivative with an absorption peak at 560 nm. By measuring the production of urea, the size of the arginine activity can be calculated

Reagents and Equipment Required but Not Provided:

Visible spectrophotometer/enzymometer, cryogenic centrifuge, water bath/incubator, analytical balance,

adjustable pipettes, micro-glass cuvettes/96-well plates, mortar and pestle/homogenizer/cellular ultrasonic breakers, distilled water and ice.

I. Sample extraction:

1. Tissue:

Accordance the ratio of tissue(g) : extract solution volume (mL)=1: 5~10 (add 1 mL of extract solution to 0.1 g of tissue), homogenate on ice. Centrifuge at 12000g for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

2. Bacteria or cells:

Accordance the ratio of cells amount(10^4) : extract solution volume (mL)=5~10: 1 (add 1 mL of extract solution to 5 million cells). Ultrasonic on ice bath to smash cells, (powder 200w, ultrosonic 3s, interval 10s for 5 minutes). Centrifuge at 12000g for 10 minutes at 4℃, take the supernatant and place it on ice for testing.

II. Determination procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 560 nm, spectrophotometer set the counter to zero with distilled water.
2. Preparation of standard solutions: The standards were diluted with distilled water to 50, 25, 12.5, 6.25 and 3.125 μmol/mL respectively.
3. Add the following reagents: (In 1.5m LEP tubes follow the steps in the table below)

Reagent name （μL）	Test tube (T)	Contrast tube (C)	Standard tube (S)	Blank tube (B)
Sample	48	48	-	-
Standard	-	-	48	-
Distilled water	-	-	-	48
Reagent I	24	24	24	24
Reagent III	72	72	72	72
Reagent IV	-	96	96	96
30min at 37°C protected from light				
Reagent IV	96	-	-	-
Centrifuge at 8000g for 5min at room temperature, take another 1.5mLEP and aspirate 1000μL of supernatant into an EP tube				
Supernatant	200	200	200	200
Reagent V	40	40	40	40
The reaction was boiled in boiling water and protected from light for 40min.The supernatant was centrifuged at 8000g for 5min at room temperature and the absorbance at 560nm was measured in a cuvette and recorded as A_T, A_C, A_S and A_B. $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$ (Standard and blank tubes should only be done 1-2 times, one control tube per assay tube is required.)				

III. Calculation of Arginase activity:

1 Make standard curve:

A standard curve was established based on the concentration of the standard tube (x, μmol/mL) and the absorbance ΔAs (y, ΔAs). Based on the standard curve, the ΔAt was substituted into the equation to obtain x (μmol/mL).

2 Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 μmol urea per min every. mg tissue protein

Arginase activity（U/mg prot）= x×Vs÷（Cpr×Vs）÷T×F= x÷30÷Cpr×F

3 Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 μmol urea per min every gram tissue weight.

Arginase activity （U/g weight）= x ×Vs÷（N÷V_E×Vs）÷T×F= x÷30÷N×F

4 Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 μmol urea per min every 10^6 cells.

Arginase activity （U/10^6 cell）= x×Vs÷（N÷V_E×Vs）÷T×F= x÷30÷N×F

Vs：Volume of sample added to the reaction system，0.048mL；V_E：Volume of extraction solution added，1mL；T：Reaction time，30min；Cpr：Protein concentration，mg/mL；W：Sample quality，g；N：Number of cells or bacteria，In units of 10^6；F：Sample dilution。

Note:

1. If the measured absorbance value is greater than 1.5 or ΔA_T is greater than 1, the sample can be diluted or the first step of the 37°C reaction time can be shortened; if the measured absorbance value or ΔA_T is too small, the sample size can be increased or the first step of the 37°C reaction time can be extended. Simultaneously modify the formulae for the final calculation.

Experimental Examples:

1. Weigh 0.1198g of mouse kidney tissue, add the extract for ice bath homogenization, operate according to the assay procedure, measure with a 1mL glass cuvette to calculate $\Delta A_{T=}$ A_{T-} Ac =0.425-0.102=0.323, bring into the standard curve y=0.0168x-0.0541（R^2 = 0.9941）， x=22.446 μmol/mL, brought into the equation to calculate

Arginase activity (U/g mass) = x÷30÷W×F =6.245U/g mass

2. Weigh 0.0989 g of carrot, add the extract to the ice bath and homogenize, follow the assay procedure and measure with a 1 mL glass cuvette to calculate $\Delta A_{T=}$ A_{T-} Ac =0.086-0.063=0.023, bring into the standard curve y=0.0168x-0.0541（R^2 = 0.9941）， x=4.5893μmol/mL, and Brought into the equation to calculate:

Arginase activity (U/g mass) = x ÷ 30 ÷ W x F =1.547 U/g mass

References：

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[2] Ishii N, Ikenaga H, Carmines P K, et al. High glucose augments arginase activity and nitric oxide

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[3] Zharikov S, Krotova K, Hu H, et al. Uric acid decreases NO production and increases arginase activity in cultured pulmonary artery endothelial cells[J]. American Physiological Society, 2008(5).