Arginine (Arg) content Assay Kit

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

Operation Equipment: Microplate Reader/ Spectrophotometer

Catalog Number: BC5635

Size: 100T/96S

Components:

Extract I: Liquid 110 mL ×1. Storage at 2-8℃.

Extract II: Liquid 17 mL ×1. Storage at 2-8℃.

Reagent I: Powder ×1. Store at 2-8℃. Add 0.6mL of anhydrous ethanol before use and fully dissolve. Unused reagents are stored in separate packages and can be stored at -20℃ for up to 4 weeks.

Reagent II: Liquid 6 mL×1. Storage at 2-8℃.

Working solution preparation: according to the sample volume in accordance with the ratio of reagent I: reagent II= 10 μL: 90 μL (100 μL, 2T), prepare before use.

Reagent III: Liquid 6 mL×1. Storage at 2-8℃.

Reagent IV: Liquid 6mL×1. Storage at 2-8℃.

Standard: Powder×1. Storage at 2-8℃. Add 0.918 mL of distilled water and fully dissolve to make 62.5 μmol/mL arginine standard solution. Before use, take 10 μL of 62.5 μmol/mL arginine standard solution in EP tube, add 790 μL of distilled water to fully dissolve, and prepare 0.78125 μmol/mL arginine standard solution.

Product Description

Arginine is a semi-essential amino acid in human body and animal body, and plays an important physiological role in protein synthesis and metabolism, as well as the synthesis of polyamine and NO in the organism. Arginine has the effect of lowering blood pressure, in vivo arginine can be decomposed into nitric oxide, nitric oxide can relax the smooth muscle of the blood vessel wall, regulate the elasticity of blood vessels, and have a restorative effect on the endothelium. Arginine can stimulate and induce the secretion of adrenal hormones, thus lowering blood sugar and reducing the production of fatty acids in the body, which can bring down the blood sugar of hyperglycemic patients to normal level.

Arginine content is calculated from its characteristic absorption peak at 525 nm in alkaline medium with cresol and sodium hypochlorite to produce a red product.

Reagents and Equipment Required but Not Provided.

Microplate Reader/ Spectrophotometer, cryogenic centrifuge, water bath/constant temperature incubator, vortex mixer, analytical balance, adjustable pipette, micro glass cuvettes/96-well plates, mortar/homogenizer/cell ultrasonic breaker, distilled water, anhydrous ethanol and ice.

Procedure

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I. Sample preparation:

1. Tissue: according to the mass (g): extract I volume (mL) of 1:5~10 ratio (it is recommended to weigh about 0.1g, add 1mL of extract I) add extract I, ice bath homogenization, centrifuged at 4℃, 12000g for 10min, take 0.8mL supernatant, and then slowly add 0.15mL extract II, slowly blowing and mixing until no bubbles, 4℃. After centrifugation at 12000g for 10min, take the supernatant and leave it for measurement.
2. Cells: according to the number of cells (10^6): the volume of extract I (mL) for the ratio of 5 ~ 1: 1 (it is recommended that 5 million cells to add 1mL of extract I), ice bath ultrasonic cell crushing (power of 300w, ultrasonic 3 seconds, interval of 7 seconds, the total time of 3min); at 4℃, 12,000g centrifugation for 10min, 0.8mL of supernatant, then slowly add 0.15mL extract II,, slowly blowing mixed until no bubbles are generated, 4 ℃, 12000g centrifugation 10min and take the supernatant to be measured. mL of extract two, slowly blowing and mixing until no bubbles, 4 ℃, 12000g centrifugation for 10min, and then take the supernatant to be measured.
3. Serum (plasma) and other liquids: take 100μL of liquid and add 1mL of extract I, centrifuge at 4℃ 12000g for 10min, take 0.8mL of supernatant, and then slowly add 0.15mL of extract II, slowly blowing and mixing until no bubbles are generated, centrifuge at 4℃ 12000g for 10min, and then take the supernatant to be measured.

Note: Extraction reagent II should be added slowly, after addition will produce a lot of bubbles, it is recommended to use 2mL EP tube for operation.

II. Determination Procedure

1. Preheat the microplate reader/spectrophotometer for more than 30 minutes, adjust the wavelength to 525 nm and spectrophotometer set the counter to zero with distilled water.
2. Add samples in 1.5 mLEP tubes according to the following table:

Reagent Name (μL)	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	50	-	-
Standard	-	50	-
Distilled water	-	-	50
Working solution	50	50	50
Protect from light, ice bath reaction for 20min			
Reagent III	50	50	50
Full shock for 30s			
Reagent IV	50	50	50
After thorough mixing, **ice bath reaction for 2min**. The absorbance at 525 nm was measured in a cuvette and recorded as A_T, As, Ab. $\Delta A_T = A_T - Ab$, $\Delta As = As - Ab$. (Standard and blank tubes should only be done 1-2 times)			

III. Calculation:

1. Calculate by protein content

Arg content（μmol/mg prot）=$C_S \times \Delta A_T \div \Delta A_S \times V_S \div$（$Cpr \times V_S$）$\times F = 0.781 \times \Delta A_T \div \Delta A_S \div Cpr \times F$

2. Calculate by sample weight

Arg content（μmol/g weight）= $C_S \times \Delta A_T \div \Delta A_S \times$（$V_{SU}+V_{E2}$）÷（$W \times V_{SU} \div V_{E1}$）$\times F$

$= 0.928 \times \Delta A_T \div \Delta A_S \div W \times F$

3. Calculate by number of bacteria or cells

Arg content（μmol/10^6 cell）= $C_S \times \Delta A_T \div \Delta A_S \times$（$V_{SU}+V_{E2}$）÷（$N \div V_{SU} \div V_{E1}$）$\times F$

$= 0.928 \times \Delta A_T \div \Delta A_S \div N \times F$

4. Calculate by liquid volume

Arg content（μmol/mL）= $C_S \times \Delta A_T \div \Delta A_S \times$（$V_{SU}+V_{E2}$）÷（$V_L \times V_{SU} \div$（$V_L+V_{E1}$））$\times F$

$= 10.205 \times \Delta A_T \div \Delta A_S \times F$

C_S: Arginine standard solution concentration, 0.78125 μmol/mL;V_s：Volume of sample added to the reaction system，0.05mL；V_{su}：Volume of supernatant at extraction，0.8mL；V_{E2}：Add volume of extract II，0.15mL；V_{E1}：Volume of Extract I added，1mL；V_L：Liquid sample volume，0.1mL；Cpr：protein concentration，mg/mL；W：sample quality，g；N：Number of cells or bacteria，10^6；F：Sample dilution.

Note：

1. If the ΔA_T is greater than 1.2, the sample can be diluted with distilled water; if the ΔA_T is less than 0.01, the sample size can be increased. Modify the formula synchronously for the final calculation.
2. Extract I contains protein precipitant, so the supernatant cannot be used for protein concentration determination. If protein content is to be determined, a separate tissue is required.

Experimental example:

1. Weigh 0.1162g of mouse kidney tissue, add the extraction solution for ice bath homogenization, operate according to the assay steps, measured with a 1mL glass cuvette to calculate the $\Delta A_T = A_T - Ab = 0.524-0.232=0.292$, $\Delta As = As - Ab = 0.575-0.232=0.343$, brought to the formula:
   Arg content (μmol/g mass) = $0.928 \times \Delta A_T \div \Delta As \div W = 6.799$ μmol/g mass.
2、 Weigh 0.1186g of peanut tissue, add the extraction solution for ice bath homogenization, according to the steps of the assay, measured with a 1mL glass cuvette to calculate the $\Delta A_T = A_T - Ab = 0.556-0.232=0.324$, $\Delta As = As - Ab = 0.575-0.232=0.343$, carried into the formula calculation:
   Arg content (μmol/g mass) = $0.928 \times \Delta A_T \div \Delta As \div W = 7.391$ μmol/g mass.

3、Take human serum according to the pre-treatment and experimental steps of the experiment, measured with a 1mL glass cuvette to calculate $\Delta A_T = A_T - Ab = 0.3-0.232=0.068$, $\Delta As = As - Ab = 0.575-0.232=0.343$, brought into the formula:

Arg content (μmol/mL) = $10.205 \times \Delta A_T \div \Delta As \times F = 2.023$ μmol/mL.

References:

[1] Francis P S, Barnett N W, Foitzik R C, et al. Chemiluminescence from the Sakaguchi reaction [J]. Analytical Biochemistry, 2004, 329(2):340-341.

Related Products:

BC1500/BC1505	Plant Nitrate Nitrogen Assay Kit
BC1520/BC1525	Plant Ammoniacal Nitrogen Assay Kit
BC1530/BC1535	Urea Nitrogen (BUN) Assay Kit
BC4370/BC4375	Plant Ureide Content Assay Kit
BC5550/BC5555	Arginase Activity Assay Kit