CheKine™ Micro Arginine (Arg) Content Assay Kit

Cat #: KTB1411 Size: 48 T/48 S 96 T/96 S

	Micro Arginine (Arg) Content Assay Kit		
REF	**Cat #:** KTB1411	LOT	**Lot #:** Refer to product label
	Detection range: 31.25-1,000 nmol/mL		**Sensitivity:** 31.25 nmol/mL
	Applicable samples: Animal and Plant Tissues, Bacteria, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Arginine is a semi-essential amino acid in the human and animal bodies. It participates in the anabolicity of proteins and the synthesis of polyamines and nitric oxide in the body, and plays an important physiological role. Arginine has the effect of lowering blood pressure. In the body, arginine can decompose into nitric oxide. Nitric oxide can relax the smooth muscle of the blood vessel wall, regulate the elasticity of blood vessels, and repair the endometrium of the blood vessels. Arginine can stimulate and induce adrenal stimulation secretion, thereby reducing blood sugar, reducing the production of fatty acids in the body, and reducing the blood sugar of patients with hyperglycemia to normal levels. CheKine™ Micro Arginine (Arg) Content Assay Kit can be used to detect biological samples such as animal and plant tissues, bacteria, cells, plasma, serum or other liquid samples. In the kit, Arg produces a red product with α-naphthol and 2,3-butanedione in an alkaline medium, which has a characteristic absorption peak at 525 nm. The content of Arg can be calculated by measuring the absorbance of this wavelength.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	60 mL	120 mL	4℃
Extraction Buffer Ⅱ	10 mL	20 mL	4℃
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	Powder×2 vials	Powder×2 vials	4℃, protected from light
Reagent Ⅲ	100 μL	200 μL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 525 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water, PBS, anhydrous ethanol
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer Ⅰ : Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Extraction Buffer Ⅱ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent Ⅰ: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Working Reagent Ⅱ: Prepared before use. Add 682.5 μL anhydrous ethanol, 17.5 μL Reagent Ⅲ to each Reagent Ⅱ for 48 T, and 1365 μL anhydrous ethanol, 35 μL Reagent Ⅲ to each Reagent Ⅱ for 96 T to fully dissolve. Working Reagent Ⅱ is freshly prepared.

Reagent Ⅲ: Ready to use as supplied; Store at 4℃, protected from light.

Note: Working Reagent Ⅱ or Reagent Ⅲ has certain irritation, so personal protection is recommended during use.

Standard: Prepared before use. Add 1 mL deionized water to fully dissolve, that is 50 μmol/mL Arg Standard; Equilibrate to room temperature before use; Store at 4℃ for 1 month. Using 50 μmol/mL Arg Standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (nmol/mL)
Std.1	20 μL of 50 μmol/mL Standard	980	1,000
Std.2	100 μL of Std.1 (1,000 nmol/mL)	100	500
Std.3	100 μL of Std.2 (500 nmol/mL)	100	250
Std.4	100 μL of Std.3 (250 nmol/mL)	100	125
Std.5	100 μL of Std.4 (125 nmol/mL)	100	62.5
Std.6	100 μL of Std.5 (62.5 nmol/mL)	100	31.25
Blank	0	100	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for 1 month.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer Ⅰ and homogenize or mortar on ice. Centrifuge at 12,000 g for 10 min at 4℃. Take 800 μL supernatant, add 150 μL Extraction Buffer Ⅱ and mix well slowly. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.
2. Bacteria or cells: Collect 5×10^6 bacteria or cells into the centrifuge tube, wash bacteria or cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer Ⅰ to ultrasonically disrupt the bacteria or cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 12,000 g for 10 min at 4℃. Take 800 μL supernatant, add 150 μL Extraction Buffer Ⅱ and mix well slowly. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.
3. Plasma, Serum or other Liquid samples: Take 100 μL liquid sample, add 1 mL Extraction Buffer Ⅰ and mix well. Centrifuge at 12,000 g for 10 min at 4℃. Take 800 μL supernatant, add 150 μL Extraction Buffer Ⅱ and mix well slowly. Centrifuge at 12,000 g for 10 min at 4℃. Use supernatant for assay.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample. Because Extraction Buffer Ⅰ and Extraction Buffer Ⅱ can cause protein degeneration, the sample needs to be extracted with

deionized water according to the steps separately.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 525 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Reagent Ⅰ	130	130	130
Sample	0	0	50
Standard	0	50	0
Deionized Water	50	0	0
Working Reagent Ⅱ	20	20	20

3. Mix well, incubate for 20 min at 30℃, the absorbance value is measured at at 525 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1,000 nmol/mL of $\Delta A_{Standard}$, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get x (nmol/mL).

2. Calculation of the Arg content

(1) Calculated by protein concentration

Arg (nmol/mg prot)=$V_{Sample}\times x\div(V_{Sample}\times Cpr)\times F$**=$x\div Cpr\times F$**

(2) Calculated by fresh weight of samples

Arg (nmol/g fresh weight)=$(V_{Supernatant}+V_{Extr\ II})\times x\div(w\times V_{Supernatant}\div V_{Extr\ I})\times F$**=$1.1875x\div w\times F$**

(3) Calculated by number of bacteria or cells

Arg (nmol/10^6)=$(V_{Supernatant}+V_{Extr\ II})\times x\div(n\times V_{Supernatant}\div V_{Extr\ I})\times F$**=$1.1875x\div n\times F$**

(4) Calculated by volume of liquid samples

Arg (nmol/mL)=$(V_{Supernatant}+V_{Extr\ II})\times x\div[V_{Liquid}\times V_{Supernatant}\div(V_{Liquid}+V_{Extr\ I})]\times F$**=$13.0625x\div n\times F$**

V_{Sample}: Added the sample volume, 0.05 mL; $V_{Supernatant}$: Volume of supernatant during extraction, 0.8 mL; $V_{Extr\ I}$: Added Extraction Buffer Ⅰ volume, 1 mL; $V_{Extr\ II}$: Added Extraction Buffer Ⅱ volume, 0.15 mL; V_{Liquid}: Volume of liquid sample, 0.1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; n: Number of bacteria or cells, calculated in units of one million; F: Sample dilution multiple.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

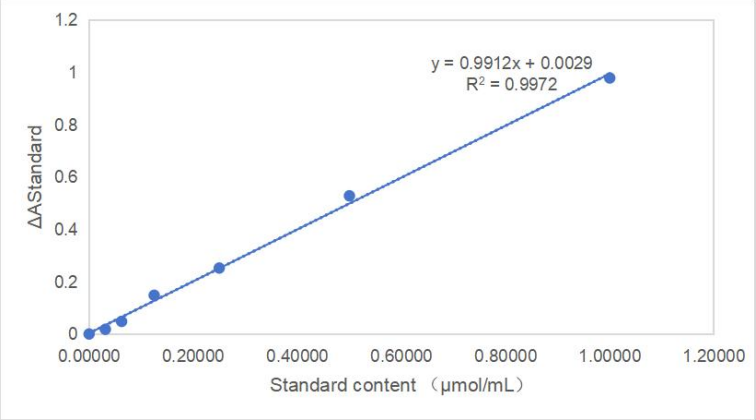

Figure 1. Standard curve of Arg.

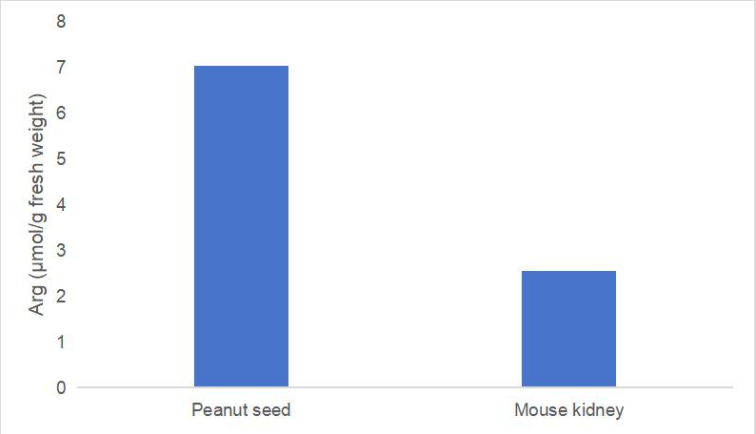

Figure 2. Determination of Arg in peanut seed and mouse kidney by this kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.