（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K848-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(500-540 nm)

Detection range：0.003-0.3 U/L

Elabscience® Arginase Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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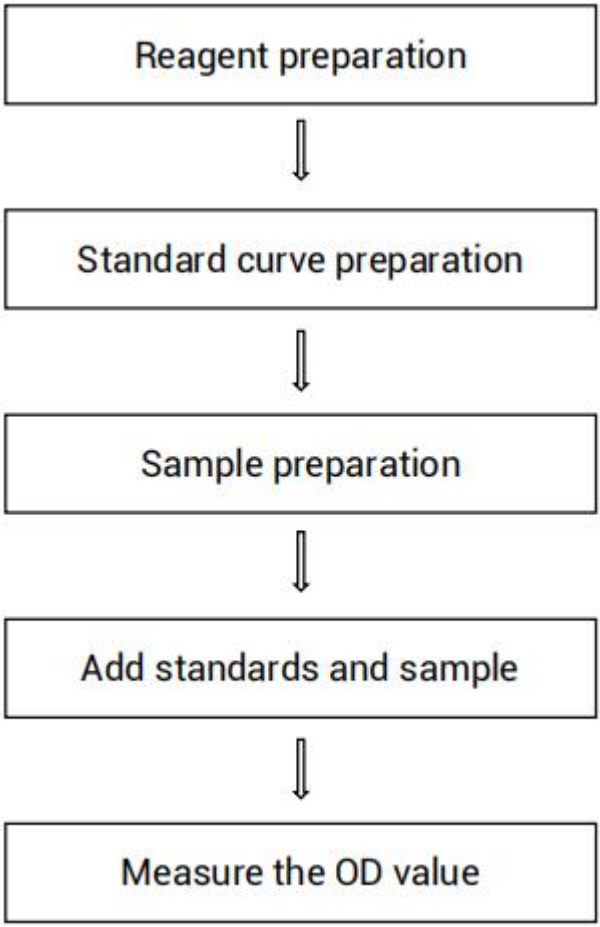
Assay summary



Intended use

This kit can measure arginase activity in tissue and cell samples.

Detection principle

Arginase is a key enzyme in the urea cycle. Arginase plays an important role in tumor, cardiovascular disease and so on. The principle of this kit is that arginase catalyzes substrate decomposition to produce urea, and urea reacts with chromogenic agent to produce chromogenic substance. The optimal detection wavelength of this product is 520 nm. By detecting the amount of chromogenic substance produced per unit time, the activity of arginase in the sample can be calculated.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	10 mL × 1 vial	-20°C, 12 months
Reagent 2	5 mmol/L Standard	1.8 mL × 2 vials	-20°C, 12 months
Reagent 3	Chromogenic Agent A	50 mL × 1 vial	-20°C, 12 months
Reagent 4	Chromogenic Agent B	25 mL× 1 vial	-20°C, 12 months, shading light
Reagent 5	Chromogenic Stock Solution	0.6 mL × 1 vial	-20°C, 12 months
Reagent 6	Saline Solution	0.5 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (500-540 nm, optimum wavelength: 520 nm), Incubator(37℃), Water bath

Reagents:

Normal saline (0.9% NaCl), Deionized water

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of chromogenic agent B working solution:
Take a piece of chromogenic stock solution, pour all of it into chromogenic agent B, and mix well. It is recommended that chromogenic agent B working solution be prepared after opening the kit and can be stored at -20℃ for 1 month.

③ The preparation of chromogenic working solution:
For each well, prepare 450 μL of chromogenic working solution (mix well 300 μL of chromogenic agent A and 150 μL of chromogenic agent B working solution). Store at -20°C for 4 days protected from light.

④ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions after use.
Dilute 5 mmol/L standard with deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 2.5, 3, 3.5, 4, 5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**1**	**2**	**2.5**	**3**	**3.5**	**4**	**5**
5 mmol/L Standard (μL)	0	40	80	100	120	140	160	200
Deionized water (μL)	200	160	120	100	80	60	40	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse liver tissue homogenate	20-120
1×10^6 293T cells	1
1×10^6 HepG2 cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① According to the activity of arginase in different samples, the appropriate dilution ratio was selected. If the urea content in the sample is too high and the reaction system is orange-yellow after a 95℃ water bath, the dilution ratio should be increased.

② After the 95℃ water bath is finished, it needs to be cooled to room temperature and then added to the microplate for detection.

Operating steps

① Sample pretreatment: Before testing, take 5 μL of saline solution and 500 μL of samples, mix fully.

② Standard tube: Add 25 μL of standard solution with different concentrations into the corresponding tube.
Sample tube: Add 25 μL of sample into sample tube.
Control tube: Add 25 μL of sample into control tube.

③ Add 75 μL of buffer solution into sample tube. Incubate at 37℃ for 15 min.

④ Add 450 μL of chromogenic working solution into each tube.

⑤ Add 75 μL of buffer solution into standard and control tubes.

⑥ Incubate in 95°C water bath for 10 min. Cool the tubes to room temperature and take 200 μL of supernatant to the microplate. Measure the OD values of each well at 520 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue and cell samples:

Definition: The amount of enzyme in 1 g sample protein that hydrolyze the substrate to produce 1 mmol urea in 1 minute at 37°C is defined as 1 unit.

$$\frac{\text{ARG activity}}{\text{(U/gprot)}} = (\Delta A - b) \div a \div t \div Cpr \times f$$

[Note]

ΔA: $\Delta A = OD_{Sample} - OD_{Control}$.

T: the time of tissue sample reaction, 15 min.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse kidney samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.50	10.00	15.00
%CV	3.1	2.5	4.8

Inter-assay Precision

Three mouse kidney samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.50	10.00	15.00
%CV	7.2	8.1	10.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 102.7%.

	Standard 1	Standard 2	Standard 3
Expected Conc.（U/L）	2.5	10	15
Observed Conc.（U/L）	2.5	10.3	15.75
recovery rate(%)	100	103	105

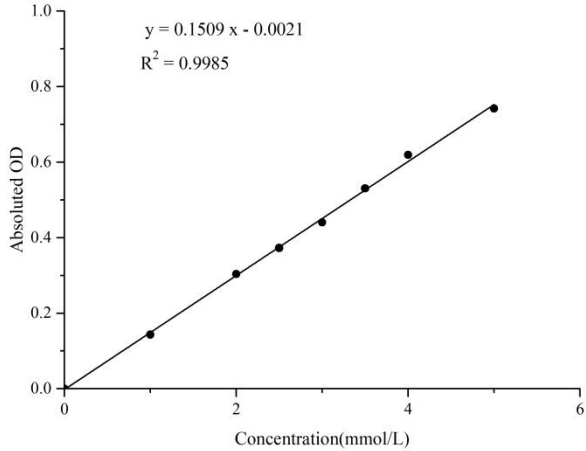
Sensitivity

The analytical sensitivity of the assay is 0.003 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	0	1	2	2.5	3	3.5	4	5
OD Value	0.053	0.194	0.355	0.423	0.484	0.581	0.666	0.786
	0.052	0.198	0.358	0.428	0.502	0.585	0.678	0.803
Average OD	0.052	0.196	0.356	0.425	0.493	0.583	0.672	0.794
Absoluted OD	0	0.143	0.304	0.373	0.440	0.530	0.619	0.742



Appendix Π Example Analysis

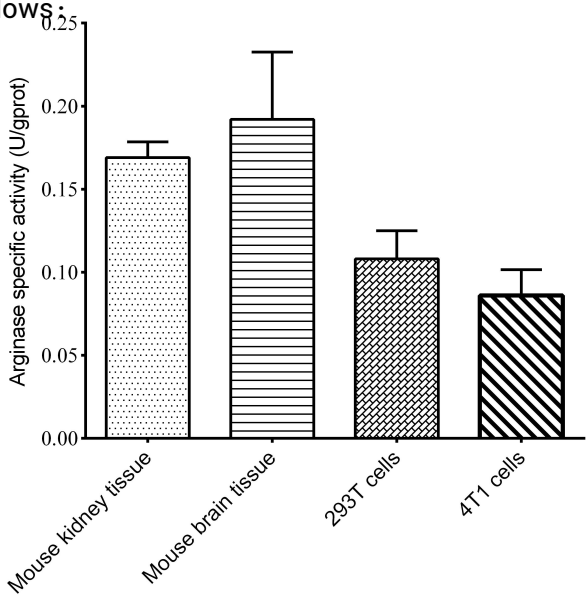
Example analysis：

Take 25 μL of 10% mouse liver tissue homogenate which dilute for 100 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.1401 x – 0.0094, the average OD value of the sample is 0.373, the average OD value of the control is 0.081, ΔA = OD_{Sample} – $OD_{control}$ = 0.373 – 0.081 = 0.292, and the calculation result is:

$$\text{ARG activity (U/gprot)} = (0.373 - 0.081 + 0.0094) \div 0.1401 \div 15 \div 3.18 \times 100 = 4.15 \text{ U/gprot}$$

Detect 10% mouse kidney tissue homogenate (the concentration of protein is 3.28 gprot/L), 10% mouse brain tissue homogenate (the concentration of protein is 1.40 gprot/L), 1×10^6 293T cells (the concentration of protein is 0.26 gprot/L), 1×10^6 4T1 cells (the concentration of protein is 0.26 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.