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

Catalog No：E-BC-K896-M

Specification：48T (23 samples)/96T (47 samples)

Measuring instrument：Microplate reader (293 nm)

Detection range：0.06-4.00 U/L

Elabscience® Uricase Acitivity 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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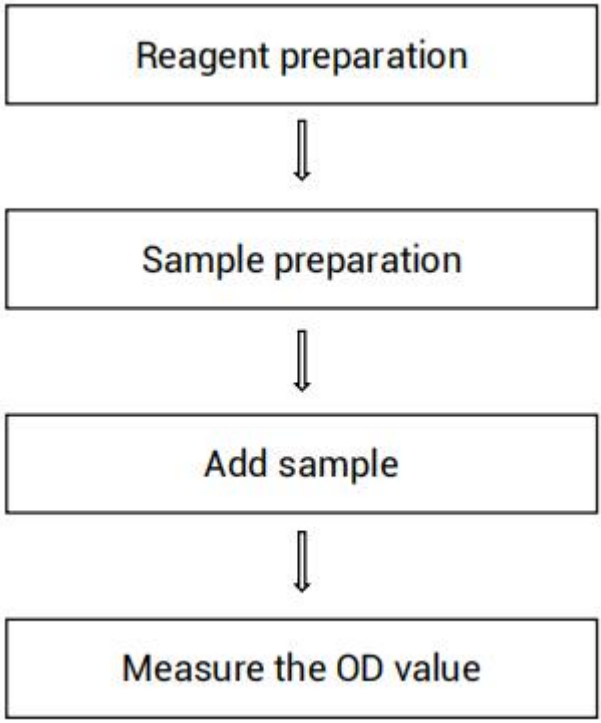
Assay summary



Intended use

This kit can measure uricase activity in animal and plant tissue samples.

Detection principle

Uricase is an enzyme in the purine decomposition pathway, which can specifically catalyze the oxidation of uric acid into allantoin with higher solubility, thereby reducing the blood uric acid level.

The detection principle of this kit is: uricase can oxidize the substrate, and the substrate has the maximum absorption peak at 293 nm. The uricase activity was calculated by measuring the OD value reduction at 293 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	35 mL × 1 vial	35 mL × 2 vials	-20°C, 12 months, shading light
Reagent 2	Substrate	Powder × 4 vials	Powder × 8 vials	-20°C, 12 months, shading light
Reagent 3	Matrix Solution	6 mL × 1 vial	12 mL × 1 vial	-20°C, 12 months, shading light
	UV-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 (optimum wavelength: 293 nm), Incubator, Ultrasonic cleaner

Reagents:

PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of substrate working solution:
Dissolve one vial of substrate with 1 mL of matrix solution, mix well to dissolve by ultrasonic cleaner. Store at -20°C for 3 days.

③ The preparation of working solution:
Before testing, please prepare sufficient working solution. For example, prepare 150 μL of working solution (mix well 5 μL of substrate working solution and 145 μL of buffer solution). The working solution should be prepared on spot and used up within 8 hours.

④ The preparation of reaction working solution:
For each well, prepare 160 μL of reaction working solution (mix well 32 μL of working solution and 128 μL of buffer solution). The reaction working solution should be prepared on spot and used up within 8 hours.

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 buffer solution with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared tissue supernatant within 4 h.

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1-3
10% Porcine liver tissue homogenate	1-3
10% Spinach tissue homogenate	1

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Blank well: Add 20 μL of buffer solution to blank wells.
Sample well: Add 20 μL of samples to sample wells.
Control well: Add 20 μL of samples to control wells.

② Add 160 μL of reaction working solution to blank wells and sample wells. Add 160 μL of buffer solution to control wells.

③ Mix fully with microplate reader for 5 s and measure the OD value of each well at 293 nm, as A_1.

④ Incubate at 37°C for 20 min protected from light.

⑤ Mix fully with microplate reader for 5 s and measure the OD value of each well at 293 nm, as A_2.

Calculation

The sample:

Tissue sample:

Definition: The amount of enzyme in 1 kg tissue per 1 min in a reaction system per liter that consume 1 μmol of uric acid at 37 °C is defined as 1 unit.

uricase activity
(U/Kg wet weight)

$$= (\Delta A_{sample} - \Delta A_{control} - \Delta A_{blank}) \times V_{total} \times f \div \varepsilon \div d \div V_{sample} \div T \div \frac{m}{V} \times 10\text{^}6*$$

[Note]

ΔA_{sample}: The OD value of sample wells, $A_1 - A_2$.

$\Delta A_{control}$: The OD value of control wells, $A_1 - A_2$.

ΔA_{blank}: The OD value of blank wells, $A_1 - A_2$.

f: Dilution factor of sample before test.

V_{total}: The volume of reaction system, 0.18 mL.

V_{sample}: The volume of sample added to the reaction system, 0.02 mL.

T: Reaction time, 20 min.

ε: The molar extinction coefficient, 12500 $L \cdot mol^{-1} \cdot cm^{-1}$.

d: Optical path, 0.6 cm.

m: The wet weight of sample, g.

V: The volume of buffer solution in the preparation step, mL.

10^6*: 1 mol/L = 10^6 μmol/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three mouse liver 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)	0.20	0.40	0.80
%CV	2.6	3.4	3.1

Inter-assay Precision

Three mouse liver 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)	0.20	0.40	0.80
%CV	6.7	8.8	8.1

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 99%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.20	0.40	0.80
Observed Conc. (U/L)	0.20	0.40	0.80
Recovery rate (%)	99.0	100.0	99.5

Sensitivity

The analytical sensitivity of the assay is 0.06 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.

Appendix Π Example Analysis

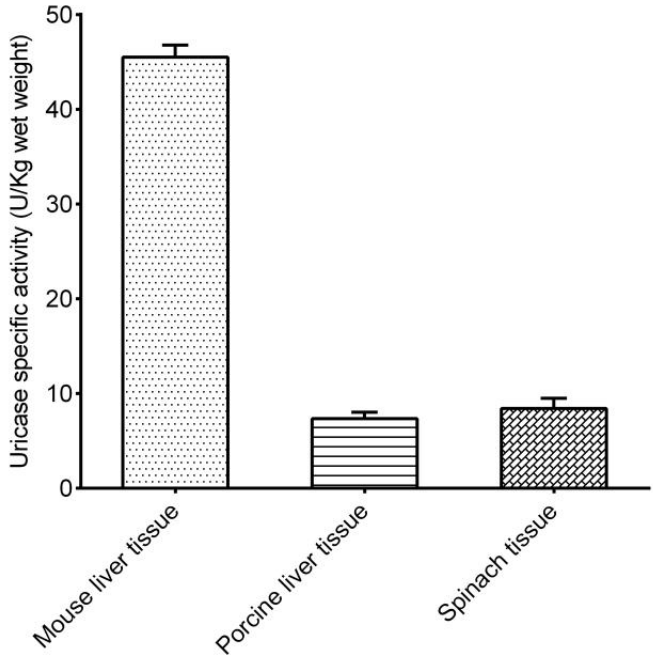
Example analysis：

Take 20 μL of 10% mouse liver tissue homogenate which dilute for 2 times and carry the assay according to the operation steps. The results are as follows:

The average A_1 of the blank well is 0.790, the average A_1 of the sample well is 1.465, the average A_1 of the control well is 0.742. The average A_2 of the blank well is 0.787, the average A_2 of the sample well is 1.395, the average A_2 of the control well is 0.716, ΔA_{sample} = 1.465 - 1.395 = 0.070，$\Delta A_{control}$ = 0.742 - 0.716 = 0.026，ΔA_{blank} = 0.790 - 0.787 = 0.003, and the calculation result is:

uricase activity (U/Kg wet weight) = (0.07 - 0.026 - 0.003) × 0.18 × 2 ÷ 12500 ÷ 0.6 ÷ 0.02 ÷ 20 ÷ 0.1× 0.9 × 1 × 10^6 = 44.28 U/Kg wet weight

Detect 10% mouse liver tissue homogenate (dilute for 2 times), 10% porcine liver tissue homogenate and 10% spinach tissue homogenate, according to the protocol, the result is as follows：


Uricase specific activity (U/Kg wet weight)
50
40
30
20
10
0
Mouse liver tissue
Porcine liver tissue
Spinach tissue

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.