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

Catalog No：E-BC-F018

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=535 nm/587 nm)

Detection range：0.03-15 μmol/L

Elabscience®Uric Acid (UA) Fluorometric 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
Tel: 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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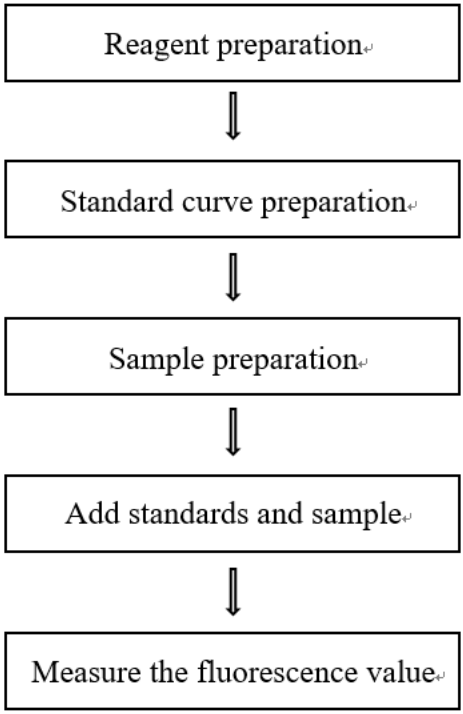
Assay summary



Intended use

This kit can be used to measure the Uric Acid (UA) content in urine, serum, plasma and animal tissue samples.

Detection principle

Uricase catalyzes the decomposition of uric acid into allantoin, CO_2 and H_2O_2. Under the action of peroxidase, H_2O_2 oxidizes the non-fluorescent probe into the fluorescent substance. By measuring the fluorescence value of the system, the corresponding uric acid content can be calculated.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	60 mL ×1 vial	60 mL ×2 vials	-20℃, 12 months
Reagent 2	Probe Solution	0.12 mL ×1 vial	0.24 mL ×1 vial	-20℃, 12 months shading light
Reagent 3	Enzyme Reagent 1	0.12 mL ×1 vial	0.24 mL ×1 vial	-20℃, 12 months
Reagent 4	Enzyme Reagent 2	0.6 mL ×1 vial	1.2 mL ×1 vial	-20℃, 12 months
Reagent 5	20 μmol/L Uric Acid Standard	1.5 mL ×1 vial	1.5 mL ×1 vial	-20℃, 12 months
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Micropipettor, Vortex mixer, Centrifuge

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of working solution：

For each well, prepare 50 uL of working solution (mix well 36 μL of buffer solution, 2 μL of probe solution, 2 μL of enzyme reagent 1 and 10 μL of enzyme reagent 2). The working solution should be prepared on spot and protected from light.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 20 μmol/L uric acid standard with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 2, 4, 6, 8, 10, 12, 15 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**2**	**4**	**6**	**8**	**10**	**12**	**15**
20 μmol/L Uric acid standard (μL)	0	20	40	60	80	100	120	150
Buffer solution (μL)	200	180	160	140	120	100	80	50

Sample preparation

① Sample preparation

Serum, plasma and other liquid sample: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 ℃ for a month.

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 to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑥ 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
Human serum	10-20
Human urine	80-100
Human hydrothorax	50-60
Rat urine	10-20
Rabbit serum	5-10
Rat serum	10-20
Porcine serum	1
10% Rat liver tissue homogenate	10-20
10% Rat kidney tissue homogenate	30-40
10% Rat lung tissue homogenate	10-20

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

Operating steps

① Standard well: add 50 μL of standard with different concentrations into the wells. Sample well: add 50 μL of sample into the wells.
② Add 50 μL of working solution into each well.
③ Mix fully with microplate reader for 5 s and incubate at 37℃ for 30 min.
④ Measure the fluorescence value at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted fluorescence 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:

1. Serum (plasma) and other liquid sample:

$$\text{UA content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue sample:

$$\text{UA content (μmol/ gprot)} = (\Delta F - b) \div a \times f \div C_{pr}$$

[Note]

ΔF: Absolute fluorescence intensity of sample ($F_{Sample} - F_{Blank}$)

f: The dilution factor of tested samples.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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 (μmol/L)	0.20	3.50	9.80
%CV	2.0	1.2	1.3

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	0.20	3.50	9.80
%CV	7.9	6.8	6.9

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 101%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	2.3	7.5	11.6
Observed Conc. (μmol/L)	2.3	7.4	12.1
Recovery rate (%)	100	99	104

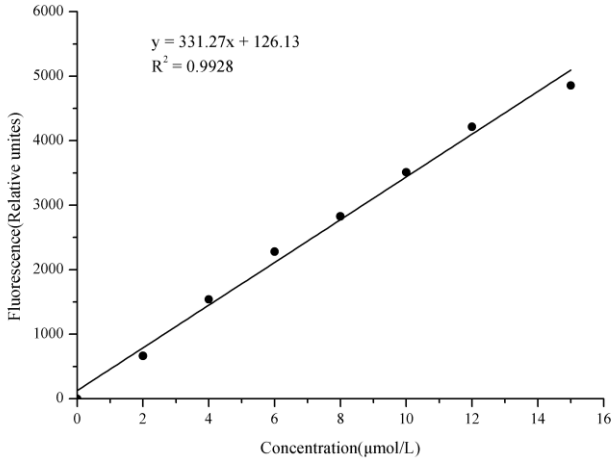
Sensitivity

The analytical sensitivity of the assay is 0.03 μmol/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 fluorescence 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 (μmol/L)	**0**	**2**	**4**	**6**	**8**	**10**	**12**	**15**
Fluorescence value	269	908	1758	2466	2974	3633	4263	4956
	263	949	1832	2626	3212	3921	4701	5285
Average fluorescence value	266	929	1805	2546	3093	3777	4482	5121
Absoluted fluorescence value	0	663	1539	2280	2827	3511	4216	4855



Appendix II Example Analysis

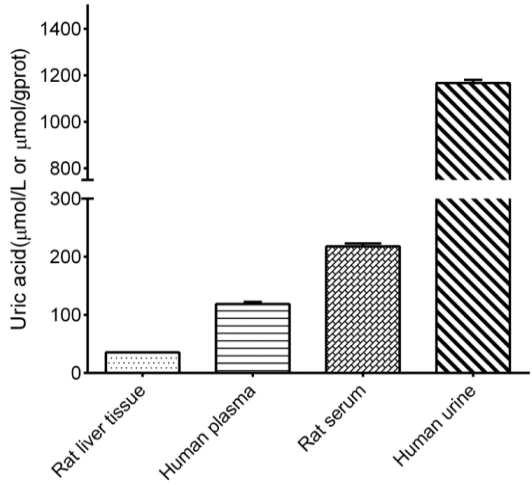
Example analysis：

Dilute 50 μL of human urine with buffer solution for 100 times, take 50 μL of diluted sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 227.73 x + 141.88, the average fluorescence value of the sample is 3077.9, the average fluorescence value of the blank is 277.3, and the calculation result is:

$$\text{UA content (μmol/L)} = (3077.9-277.3-141.88) \div 227.73 \times 100 = 1167.49 \text{ μmol/L}$$

Detect 10% rat liver tissue homogenate (the concentration of protein is 6.94 gprot/L, dilute for 20 times), human plasma (dilute for 10 times), rat serum (dilute for 20 times) and human urine (dilute for 100 times) 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.