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

Catalog No：E-BC-K897-M

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

Measuring instrument：Microplate reader (500-520 nm)

Detection range：2.95-100.00 mg/L

Elabscience® Uric Acid (UA) Colorimetric Assay Kit (Enzyme Method)

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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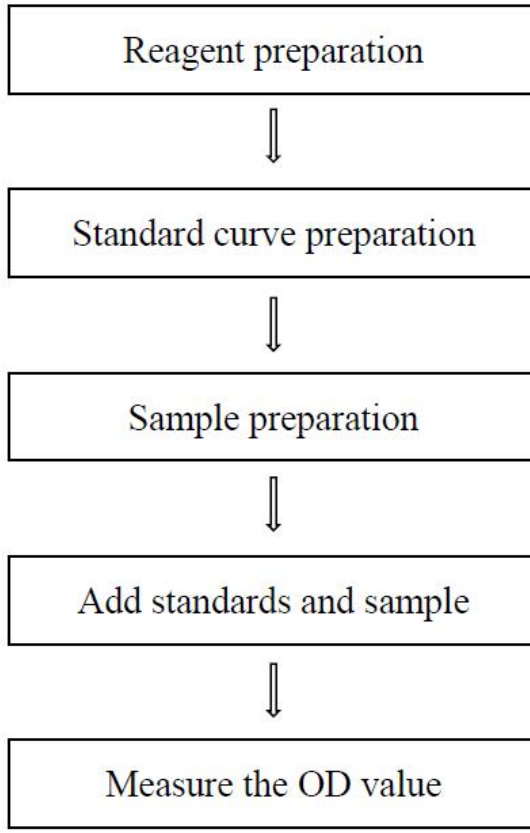
Assay summary



Intended use

This kit can be used to detect the uric acid (UA) content in serum and plasma samples.

Detection principle

Uric Acid produces allantoin, CO_2 and H_2O_2 under the action of Uricase, and H_2O_2 can produce red quinone imide compounds under the action of color developer, whose color depth is proportional to the H_2O_2 content, and has the maximum absorption at 510 nm wavelength, so as to calculate the uric acid content.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent A	12 mL × 1 vial	24 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Chromogenic Agent B	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	1 g/L Uric Acid Standard Solution	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	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:

Microplate reader (500-520 nm, optimum wavelength: 510 nm), Incubator (37°C)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of reaction working solution:

For each well, prepare 250 μL of reaction working solution (mix well 200 μL of chromogenic agent A and 50 μL of chromogenic agent A). The reaction working solution should be prepared on spot and used up within same day.

③ The preparation of 100 mg/L standard solution:

Before testing, please prepare sufficient 100 mg/L standard solution. For example, prepare 1000 μL of 100 mg/L standard solution (mix well 100 μL of 1 g/L uric acid standard solution and 900 μL of double distilled water). Store at 2-8°C for 7 days.

④ The preparation of standard curve:

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

Dilute 100 mg/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 50, 60, 80, 100 mg/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/L)	**0**	**10**	**20**	**30**	**40**	**60**	**80**	**100**
100 mg/L standard (μL)	0	20	40	60	80	120	160	200
Double distilled water (μL)	200	180	160	140	120	80	40	0

Sample preparation

① Sample preparation:

Serum (plasma) samples: detect directly.

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
Human plasma	1
Rabbit serum	1
Rat plasma	1
Bovine serum	1
Mouse plasma	1

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

Operating steps

① Standard wells: Add 5 μL of standard solution with different concentrations to the corresponding wells.

Sample wells: Add 5 μL of sample to the corresponding wells.

② Add 250 μL of reaction working solution to each well.

③ Mix fully with microplate reader for 5 s, incubate at 37°C for 10 min. Measure the OD value of each well at 510 nm.

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 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:

Serum (plasma) samples:

$$\text{UA content (mg/L)} = \frac{\Delta A - b}{a} \times f$$

[Note]

ΔA: $\Delta A = A_{sample} - A_{blank}$.

f: Dilution factor of sample before test.

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 (mg/L)	25.0	50.0	75.0
%CV	1.6	1.3	1.8

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 (mg/L)	25.0	50.0	75.0
%CV	4.2	5.1	2.7

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 94.1%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mg/L)	25.0	50.0	75.0
Observed Conc. (mg/L)	23.3	47.5	70.7
Recovery rate (%)	93.0	95.0	94.3

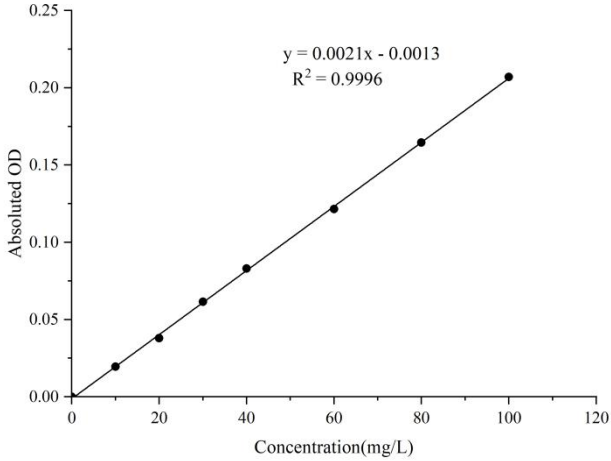
Sensitivity

The analytical sensitivity of the assay is 2.95 mg/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 (mg/L)	0	10	20	30	40	60	80	100
OD value	0.054	0.073	0.091	0.114	0.137	0.176	0.219	0.263
	0.053	0.073	0.092	0.116	0.136	0.174	0.217	0.258
Average OD value	0.054	0.073	0.092	0.115	0.137	0.175	0.218	0.261
Absoluted OD value	0.000	0.020	0.038	0.062	0.083	0.122	0.165	0.207



Appendix II Example Analysis

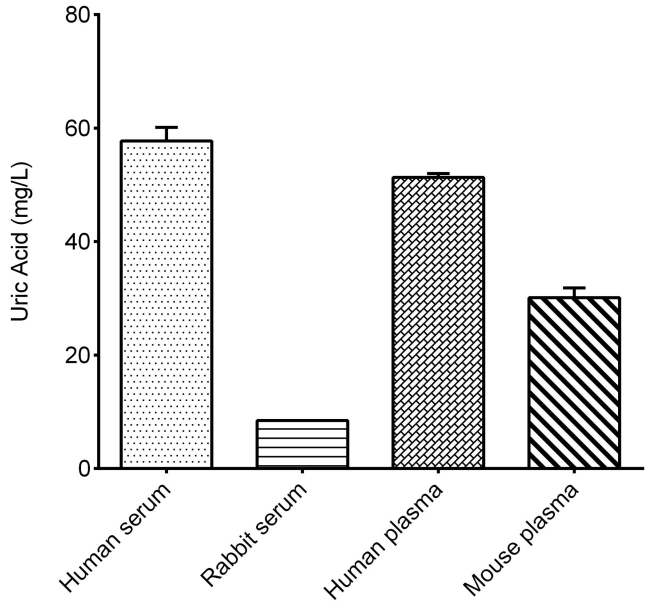
Example analysis：

Take 5 μL of human serum and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0021 x - 0.0013, the OD value of the sample well is 0.174, the OD value of the sample well is 0.054, and the calculation result is:

$$\text{UA content (mg/L)} = (0.174 - 0.054 + 0.0013) \div 0.0021 \times 1 = 57.76 \text{ mg/L}$$

Detect human serum, rabbit serum, human plasma and mouse plasma 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.