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

Catalog No：E-BC-K016-S

Specification：50 Assays (38 samples)/ 100 Assays (88 samples)

Measuring instrument：Spectrophotometer（690 nm）

Detection range：0.58-100 mg/L

Elabscience® Uric Acid (UA)

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
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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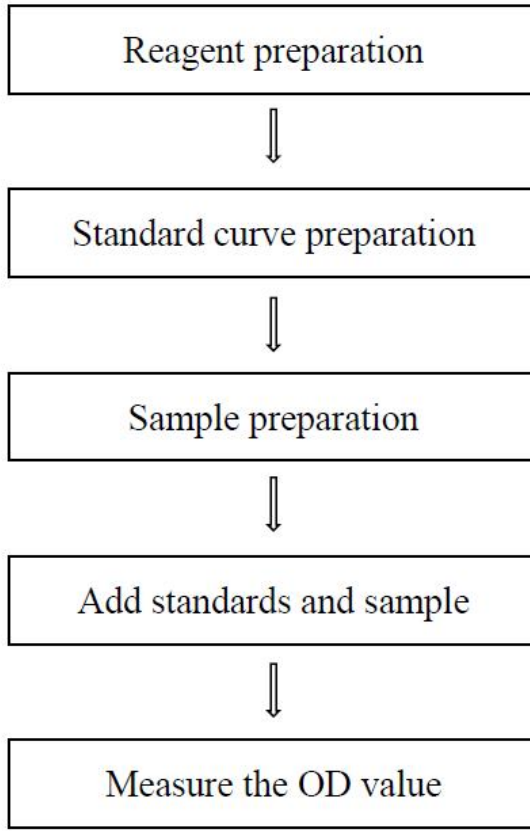
Assay summary



Intended use

This kit can be used to measure the uric acid (UA) content in serum, plasma, urine samples.

Detection principle

Uric acid can be used as an antioxidant to remove peroxide, hydroxyl and oxygen free radicals, chelate and transfer metal ions, protect vascular endothelial cells from damage. Uric Acid in protein-free filtrate reduce phosphotungstic acid to form tungsten blue, allantoin and carbon dioxide, the depth of blue color is proportional to the concentration of uric acid.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	1 g/L Uric Acid Standard	1 mL × 1 vial	1 mL × 1 vial	2-8℃, 12 months
Reagent 2	Protein Precipitator	60 mL × 2 vials	60 mL × 4 vials	2-8℃, 12 months
Reagent 3	Alkali Reagent	30 mL × 1 vial	60 mL × 1 vial	2-8℃, 12 months
Reagent 4	Phosphotungstic Acid Reagent	30 mL × 1 vial	60 mL × 1 vial	2-8℃, 12 months shading light

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 (690 nm), Micropipettor, Centrifuge, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate other reagents to room temperature before use.

② The preparation of standard curve：

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

Dilute 1 g/L uric acid standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 40, 60, 80, 100 mg/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥
Concentration (mg/L)	**0**	**10**	**40**	**60**	**80**	**100**
1g/L uric acid standard (μL)	0	10	40	60	80	100
Double distilled water (μL)	1000	990	960	940	920	900

Sample preparation

① Sample preparation

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

Urine: Collect fresh urine and centrifuge at 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

② Dilution of sample

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

Sample type	Dilution factor
Human urine	8-10
Human serum	1
Dog serum	1
Rat serum	1
Mouse serum	1-2
Porcine serum	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① The supernatant after centrifugation must be clarified.

② The color stability of uric acid is poor, so it is recommended to complete colorimetric analysis within 20 min after color development.

Operating steps

① Standard tube: add 0.2 mL of standard with different concentrations into the tubes.

Sample tube: add 0.2 mL of sample into the tubes.

② Add 2 mL of protein precipitator to each tube and mix fully with the vortex mixer.

③ Stand the tubes for 10 min. Centrifuge at 1708×g for 5 min (The supernatant should be clarified, and if turbid, transfer the supernatant into the new EP tube and centrifuge again).

④ Take 1.6 μL of the supernatant, then add 0.5 mL of alkali reagent and 0.5 mL of phosphotungstic acid reagent orderly. Mix fully and stand the tubes at room temperature for 10 min.

⑤ Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 690 nm with 1 cm optical path cuvette. Calculate $\triangle A_{690}=A_{Sample}-A_{Blank}$.

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:

$$\text{UA content (mg/L)} = (\Delta_{690} - b) \div a \times f$$

[Note]

ΔA_{530}: Absoluted OD value, $OD_{Sample} - OD_{Blank}$

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)	1.50	25.00	75.00
%CV	2.3	1.9	1.2

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)	1.50	25.00	75.00
%CV	2.4	2.7	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 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/L)	20	35	65
Observed Conc. (mg/L)	20.4	37.1	69.6
Recovery rate (%)	102	106	107

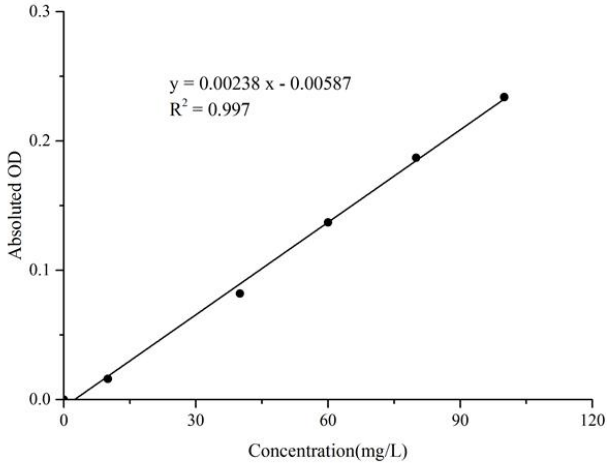
Sensitivity

The analytical sensitivity of the assay is 0.58 mg/L UA. 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**	**40**	**60**	**80**	**100**
Average OD	0.001	0.017	0.083	0.138	0.188	0.235
Absoluted OD	0	0.016	0.082	0.137	0.187	0.234



Appendix II Example Analysis

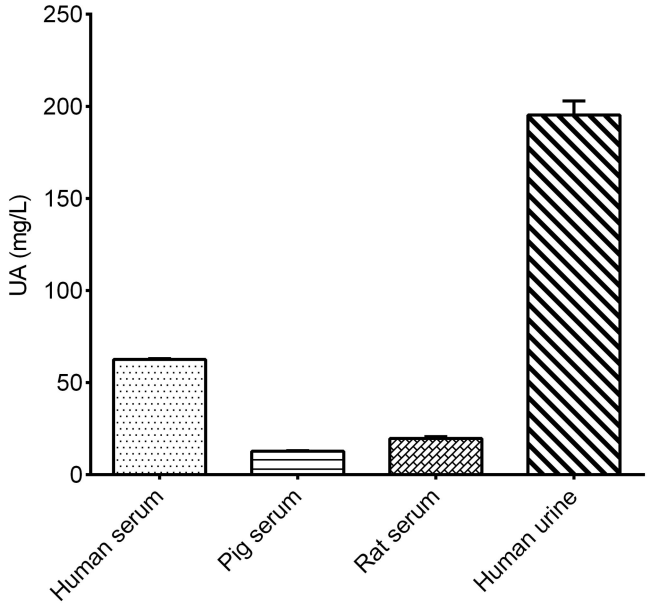
Example analysis：

For human serum, take 0.2 mL of human serum and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.0028x - 0.0112, the average OD value of the sample is 0.164, the average OD value of the blank is 0, and the calculation result is:

$$\text{Uric acid content (mg/L)} = (0.164 - 0 + 0.0112) \div 0.0028 = 62.571 \text{ mg/L}$$

Detect human serum, porcine serum, rat serum and human urine (dilute for 2 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.