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

Catalog No：E-BC-K016-M

Specification：48T(32 samples)/96T(80 samples)/500Assays(484 samples)

Measuring instrument：Microplate reader（680-700 nm）

Detection range：1.30-80 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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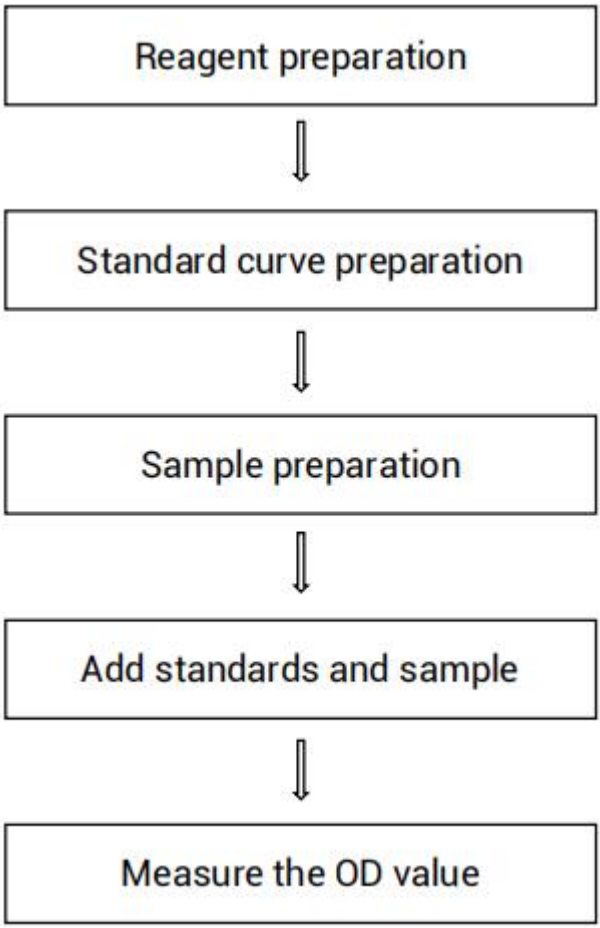
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Assay summary



Intended use

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

Detection principle

Uric acid (UA) in the protein-free filtrate reduces phosphotungstic acid to form tungsten blue, allantoin and carbon dioxide. The intensity of the blue color is proportional to the uric acid concentration. The uric acid content can be calculated by measuring the OD value at 690 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	1 g/L Uric Acid Standard	1 mL ×1 vial	1 mL × 1 vial	5 mL × 1 vial	2-8℃, 12 months
Reagent 2	Protein Precipitator	15 mL ×1 vial	30 mL×1 vial	50 mL × 3 vials	2-8℃, 12 months
Reagent 3	Alkali Reagent	3 mL ×1 vial	6 mL × 1 vial	30 mL × 1 vial	2-8℃, 12 months
Reagent 4	Phosphotungstic Acid Reagent	3 mL ×1 vial	6 mL × 1 vial	30 mL × 1 vial	2-8℃, 12 months shading light
	Microplate	48 wells	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 (680-700 nm, optimum wavelength: 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 Dilution：

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

Dilute 1 g/L Uric Acid Standard solution with deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 50, 60, 80 mg/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mg/L)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**80**
1g/L Uric Acid Standard (μL)	0	10	20	30	40	50	60	80
Deionized water (μL)	1000	990	980	970	960	950	940	920

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 minutes 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
Mouse serum	1-2
Rat serum	1
Human serum	1
Porcine serum	1
Dog serum	1-2
Human urine	8-10

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 Tungsten blue shows poor color stability; it is recommended to complete the colorimetric measurement within 20 min after color development.

Operating steps

① Standard tube: add 25 μL of standard with different concentrations into the tubes.
Sample tube: add 25 μL of sample into the tubes.

② Add 250 μL of protein precipitator to each tube and mix fully with the vortex mixer.

③ Stand the tubes for 5 min. Centrifuge at 2000 g for 5 min (The supernatant should be clarified).

④ Take 160 μL of the supernatant to the corresponding wells of microplate.

⑤ Add 50 μL of alkali reagent and 50 μL of phosphotungstic acid reagent orderly. Mix fully with microplate reader for 10 s and stand at room temperature for 15 min.

⑥ Measure the OD value of each well at 690 nm with microplate reader. (Note: Tungsten blue shows poor color stability; it is recommended to complete the colorimetric measurement within 20 min after color development).

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:

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

[Note]

ΔA_{690}: 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)	2.40	35.00	60.00
%CV	2.3	1.8	1.9

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)	0.10	0.40	0.76
%CV	4.4	2.1	0.7

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 96%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/L)	15	35	55
Observed Conc. (mg/L)	14.4	34.3	51.7
Recovery rate (%)	96	98	94

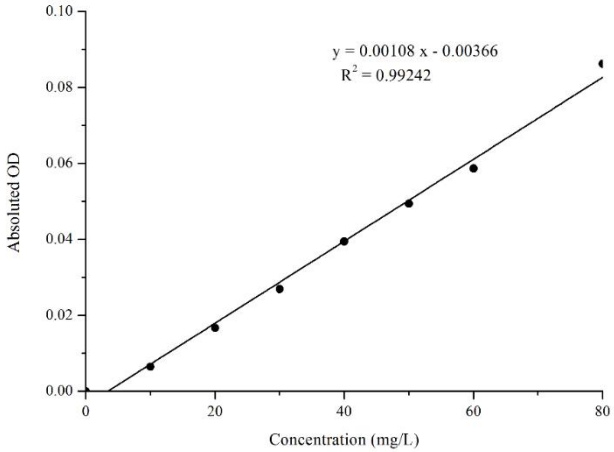
Sensitivity

The analytical sensitivity of the assay is 1.30 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	20	30	40	50	60	80
Average OD	0.037	0.043	0.053	0.064	0.076	0.086	0.095	0.123
Absoluted OD	0	0.006	0.017	0.027	0.039	0.049	0.059	0.086



Appendix Π Example Analysis

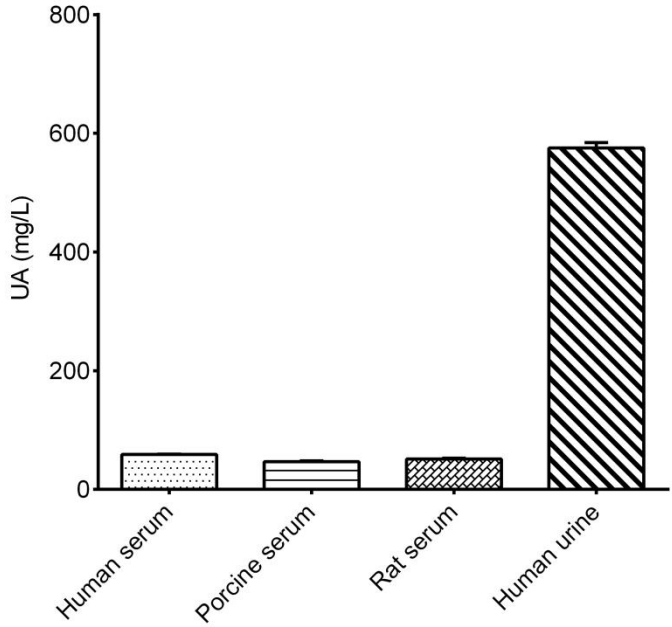
Example analysis：

For human serum, take 25 μL of human serum and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.00108 x - 0.00366, the average OD value of the sample is 0.098, the average OD value of the blank is 0.037, and the calculation result is:

UA content (mg/L) = (0.098 - 0.037 + 0.00366) ÷ 0.00108 = 59.87 mg/L

Detect human serum, porcine serum, rat serum and human urine (dilute for 10 times) according to the protocol, the result is as follows：



Appendix III Publications

1. Zeng Z, Quan C, Zhou S, et al. Gut microbiota and metabolic modulation by supplementation of polysaccharide-producing Bacillus licheniformis from Tibetan Yaks: A comprehensive multi-omics analysis[J]. International Journal of Biological Macromolecules, 2024, 254: 127808.
2. Zhang N, Zhao L, Li J, et al. Harnessing Nanotechnology for Gout Therapy: Colchicine-Loaded Nanoparticles Regulate Macrophage Polarization and Reduce Inflammation[J]. Biomaterials Research, 2024, 28: 0089.
3. Wang R, Wang H, Jiang G, et al. Transdermal delivery of allopurinol to acute hyperuricemic mice via polymer microneedles for the regulation of serum uric acid levels[J]. Biomaterials science, 2023, 11(5): 1704-1713.
4. Lin X, Zou X, Hu B, et al. Bi Xie Fen Qing Yin decoction alleviates potassium oxonate and adenine induced-hyperuricemic nephropathy in mice by modulating gut microbiota and intestinal metabolites[J]. Biomedicine & Pharmacotherapy, 2024, 170: 116022.
5. Siboto A, Akinnuga A M, Khumalo B, et al. Ameliorative Effects of a Rhenium (V) Compound with Uracil-Derived Ligand Markers Associated with Hyperglycaemia-Induced Renal Dysfunction in Diet-Induced Prediabetic Rats[J]. International Journal of Molecular Sciences, 2022, 23(23): 15400.
6. Liao Z Y, Chen P Y, Rao N V, et al. Rationally designed febuxostat-based hydroxamic acid and its pH-Responsive nanoformulation elicits anti-tumor activity[J]. European Journal of Medicinal Chemistry, 2024, 279: 116866.

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.