Uric Acid(UA) Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC1365

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Storage
Extract Solution	Solution 60 mL×1	2-8℃
Reagent I	Solution 10 mL×1	2-8℃
Reagent II	Solution 3 mL×1	2-8℃
Reagent III	Solution 10 mL×1	2-8℃
Reagent IV	Powder×1	-20℃
Standard	Solution 1 mL×1	2-8℃

Solution preparation:

Reagent IV: The powder is placed in a glass tube inside the reagent bottle. Before use, add 6 mL of Reagent I, mix thoroughly and reserve; Inexhaustible reagents are stored at -20℃ to avoid repeated freeze-thaw. Store at -20℃ for 4 weeks.

Standard Solution: 2.5 μmol/mL uric acid solution.

Working Solution A: It is prepared according to the ratio of Reagent II: Reagent III: **Reagent IV** =250μL: 750μmL: 500μL (10S) for the detection of sample determination tube, blank tube and standard tube. According to the sample size, it is recommended to use up within 2 hours after preparation (2-8℃ or stored on ice).

Working Solution B: Reagent II: Reagent III: **Reagent I** =250μL: 750μmL: 500μL (10S) is prepared for the detection of sample care. According to the sample size, it is recommended to use up within 2 hours after preparation (2-8℃ or stored on ice).

Product Description

Uric acid is the end product of purine metabolism. Disorders of purine metabolism, energy metabolism and renal excretion of uric acid can cause the increase or decrease of plasma uric acid level. And then lead to a variety of diseases such as ventilation, kidney disease, cardiovascular disease. Therefore, the determination of uric acid content has an important guiding significance in clinical diagnosis.

Uricase can catalyze the decomposition of uric acid into allantoin, CO_2 and H_2O_2. Then Fe^{2+} in potassium ferrocyanide is oxidized by H_2O_2 to form Fe^{3+}. Fe^{3+} can further react with 4-aminoantipyrine and phenol to form red Quinones, which has a characteristic absorption peak at 505 nm. The content of uric acid can be calculated by measuring the absorbance value at 505 nm.

Technical index:

Minimum detection limit: 0.00388 mg/mL

linear range: 0.003906-0.8 mg/mL

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, centrifuge, water bath/constant temperature incubator, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/ homogenizer, EP tube, ice and distilled water.

Procedure

I. Sample preparation:

1. Tissue: according to the tissue weight (g): the volume of Extract (mL) is 1:5-10. (It is recommended that add 1 mL of Extract to 0.1 g tissue). Homogenate in ice bath, then centrifuge at 10000 rpm for 10 minutes at 4℃. Take out the supernatant and put it on ice for test.
2. Bacteria or cells: By the number of bacteria/cells (10^6): Extraction liquid volume (mL) is 5~10:1. It is recommended that 5 million bacteria/cells should be added with 1.0 mL of Extract solution. Then the bacteria/cells should be crushed by ultrasound in ice bath (power 200w, ultrasonic 3s, interval 7s, total time 5minutes). Then, centrifuge at 4℃, 10000rpm, for 10minutes.
3. Serum (plasma) or urine: detect directly, If the sample is turbid, centrifugation is required.

II. Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 505 nm. The spectrophotometer needs to be zeroed with distilled water.

2. Preparation of standard solution: Dilute 2.5 μmol/mL standard solution with distilled water to 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 μmol/mL for standby. For specific dilution, please refer to the table below.

Number	Pre dilution concentration (μmol/mL)	Standard liquid volume (μL)	Volume of standard dilution solution (μL)	Diluted concentration (μmol/mL)
1	2.5	100	400	0.5
2	0.5	200	200	0.25
3	0.25	200	200	0.125
4	0.125	200	200	0.0625
5	0.0625	200	200	0.03125
6	0.03125	200	200	0.015625

Note: Each standard tube in the following experiment requires 50 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

3. Operation table: (in 1.5 mL EP	Control tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)

tube/96 well flat bottom plate) Reagent Name (μL)				
Sample	50	50	-	-
Standard	-	-	50	-
Distilled water	-	-	-	50
Working solution A	-	150	150	150
Working solution B	150	-	-	-

Mix thoroughly. React in 37℃ water bath or constant temperature incubator for 30 min. Use micro glass cuvette/96 well flat-bottom plate to measure the absorption value A at 505 nm. Record as A_C, A_T, A_S, A_B. $\Delta A_T=A_T-A_C$. $\Delta A_B=As-A_B$. Each test tube needs to set up a contrast tube, the standard curve and blank tube only need to be measured 1-2 times.

III. Calculation of UA:

1. Standard curve

According to concentration of standard solution (x， μmol/mL) and absorbance to create the standard curve, take standard solution as X-axis, ΔA(T) as Y-axis. Take ΔA(S) into the equation to obtain x (μmol/mL).

2. Calculate

(1) Calculate by sample weight

UA content (μg/g weight) $=x\times V_E\div W\times M=168x\div W$

(2) Calculate by volume

UA content (μg/mL Serum (plasma) or urine)$=x\times V_S\div V_S\times M=168x$

(3) Calculated by the number of bacteria/cells:

UA content (μg/10^6 cell) $=x\times V_E\div N\times M=168x\div N$

V_E: Extract solution volume, 1 mL;

W: Sample weight, g;

M: Molecular weight of uric acid, 168.

V_S: Sample volume, 0.05 mL;

N: The number of cells, 10^6.

Note:

1. If the absorbance value exceeds the linear range, the sample size can be increased or diluted before proceeding with the measurement. Pay attention to synchronously modifying the calculation formula.

2. Working solution A and Working solution B should be prepared when the solution will be used. It is recommended to use it within 2 hours after matching. The working fluid is light yellow. If there is discoloration, it will be regarded as failure and need to be reconfigured.

Examples:

Add 0.1g rat kidney to 1mL extract solution and grind thoroughly, centrifuge and take supernatant, follow the determination procedure to operate, with 96-well flat-bottom plates to calculate: $\Delta A_T = A_T - A_C = 0.591 - 0.347 = 0.244$, standard curve: y=1.7648x+0.0127, calculate x=0.131, according with weight of sample to calculate:

UA content (μg/g weight) =168x÷W=168×0.131÷0.1=220.08 μg/g weight.

1. Take goose serum, follow the determination procedure to operate, with 96-well flat-bottom plates to calculate: $\Delta A_T = A_T - A_C = 0.217 - 0.093 = 0.124$, standard curve: y=1.7648x+0.0127, calculate x=0.063, according with volume of sample to calculate:

   UA content (μg/ml serum) =168x=168×0.063=10.584 μg/ml serum.

Recent Product citations:

[1] Su YN, Lu PP, Yan SY, Guo XT, Ma J, Guo CX, Ma LH. Xinfuli granule alleviates metabolic remodeling through inhibition of endoplasmic reticulum stress and mitochondrial injury in heart failure. J Ethnopharmacol. 2023 Mar 1;303:115782. doi: 10.1016/j.jep.2022.115782. Epub 2022 Oct 2. PMID: 36198376.

[2] Liu HB, Yang M, Li W, Luo T, Wu Y, Huang XY, Zhang YL, Liu T, Luo Y. Dispelling Dampness, Relieving Turbidity and Dredging Collaterals Decoction, Attenuates Potassium Oxonate-Induced Hyperuricemia in Rat Models. Drug Des Devel Ther. 2023 Aug 2;17:2287-2301. doi: 10.2147/DDDT.S419130. PMID: 37551408; PMCID: PMC10404409.

[3] Cui W, Zhou H, Zhang J, Zhang J, Wu D, Rong Y, Liu F, Liu J, Liu H, Wei B, Tang Y, Liao X, Xu X. Hepatoprotective effect of Artemisia Argyi essential oil on bisphenol A-induced hepatotoxicity via inhibition of ferroptosis in mice. Environ Toxicol. 2023 Oct;38(10):2416-2428. doi: 10.1002/tox.23877. Epub 2023 Jun 22. PMID: 37347548.

[4] Li H, Zhang C, Zhang H, Li H. Xanthine oxidoreductase promotes the progression of colitis-associated colorectal cancer by causing DNA damage and mediating macrophage M1 polarization. Eur J Pharmacol. 2021 Sep 5;906:174270. doi: 10.1016/j.ejphar.2021.174270. Epub 2021 Jun 24. PMID: 34171392.

[5] Zhu Q, Miao Y, Wang J, Bai W, Yang X, Yu S, Guo D, Sun D. Isolation, identification, and pathogenicity of a goose astrovirus causing fatal gout in goslings. Vet Microbiol. 2022 Nov;274:109570. doi: 10.1016/j.vetmic.2022.109570. Epub 2022 Sep 7. PMID: 36108347.

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