Technical Manual

Uric Acid (UA) Fluorometric Assay Kit

- **Catalogue Code: MAES0005**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Fluorimetric method

Specification:

96T

Range:

0.03-15 µmol/L

Sensitivity:

0.03 µmol/L

Storage:

-20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Uric acid, a purine metabolite, is degraded into allantoin by uricase in most mammals. Due to the absence of urate oxidase gene, uric acid is the final product of purine metabolism in humans, so the level of uric acid in human blood is higher than that in most mammals. Uric acid is a physiologically important plasma antioxidant that effectively protects biological targets from the oxidation of hydroxyl radicals, hypochloric acid and peroxynitrite.

3. Intended Use

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

4. 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.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	60 mL × 2 vials	-20°C, 6 months
Probe Solution	0.24 mL × 1 vial	-20°C, 6 months, avoid direct sunlight
Enzyme Reagent 1	0.24 mL × 1 vial	-20°C, 6 months
Enzyme Reagent 2	1.2 mL × 1 vial	-20°C, 6 months
Uric Acid Standard (20 μmol/L)	1.5 mL × 1 vial	-20°C, 6 months
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Centrifuge
- Fluorescence microplate reader (Ex/Em=535 nm/587 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

The prepared working solution must be stored away from direct sunlight.

7. Reagent Preparation

1. Bring all reagent to room temperature before use.

2. The preparation of **working solution:** Mix the buffer solution, probe solution, enzyme reagent 1 and enzyme reagent 2 at a ratio of 36:2:2:10. Prepare the fresh solution before use and stored with avoid direct sunlight.

8. Sample Preparation

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve it on ice before detection.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve it on ice before detection.

3. Urine:
Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant for detection. The UA has a low solubility and is easy to form crystallization precipitation, so it should be heated to 50°C and then carry the assay.

4. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of buffer solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve it on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.03-15 μmol/L).

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	1

Note: The diluent is buffer solution.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: Ex/Em=535 nm/587 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

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

The measurement of samples

1. **Standard well:** Add 50 μL of standard with different concentrations into the corresponding wells.
   Sample well: Add 50 μL of sample into the wells. Add 50 μL of working solution into each well.

2. Mix fully with microplate reader for 5 s and incubate at 37°C for 30 min.

3. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

Operation Table

	Standard well	Sample well
Standard with different concentrations (μL)	50	
Sample (μL)		50
Working solution (μL)	50	50
Mix fully with microplate reader for 5 s and and incubate at 37°C for 30 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm**.**		

11. Calculations

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

UA content (μmol/L) = (ΔF - b) ÷ a × f

2. Tissue sample:

UA content (μmol/gprot) = (ΔF - b) ÷ a × f ÷ C_{pr}

y: The absolute fluorescence value of standard, $F_{Standard} - F_{Blank}$ (F_{Blank} is the F value when the standard concentration is 0)

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔF: The absolute fluorescence value of sample, $F_{Sample} - F_{Blank}$

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

f: Dilution factor of sample before tested

12. Performance Characteristics

Detection Range	0.03-15 μmol/L
Sensitivity	0.03 μmol/L
Average recovery rate (%)	101
Average inter-assay CV (%)	7.2
Average intra-assay CV (%)	1.5

Analysis

Dilute 50 μL of human urine with buffer solution for 100 times, take 50 μL of diluted sample, carry the assay according to the operation table.

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:

UA content (μmol/L) =（3077.9-277.3-141.88）÷ 227.73 × 100

= 1167.49 μmol/L

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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