Uric Acid (UA) Fluorometric Assay Kit

Catalog No: E-BC-F018

Method: Fluorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 0.03 μmol/L

Detection range: 0.03-15 μmol/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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.

Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 2 vials	-20°C , 6 months
Reagent 2	Probe Solution	0.24 mL × 1 vial	-20°C , 6 months, shading light
Reagent 3	Enzyme Reagent 1	0.24 mL × 1 vial	-20°C , 6 months
Reagent 4	Enzyme Reagent 2	1.2 mL × 1 vial	-20°C , 6 months
Reagent 5	20 μmol/L Uric Acid Standard	1.5 mL × 1 vial	-20°C , 6 months
	Black Microplate	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.

Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Micropipettor, Vortex mixer, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

Pre-assay preparation

▲Reagent preparation

1. Bring all the reagents to room temperature before use.
2. Preparation of working solution:
   Mix the reagent 1, reagent 2, reagent 3 and reagent 4 at the ratio of 36:2:2:10 fully. Prepare the fresh solution before use and store with shading light.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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	10-20

Note: The diluent is reagent 1.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	Ex/Em=535 nm/587 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲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.

▲Operating steps

1. The preparation of standard curve

   Dilute 20 μmol/L uric acid standard with reagent 1 to a serial concentration. The recommended dilution gradient is as follows: 15, 12, 10, 8, 6, 4, 2, 0 μmol/L.

2. The measurement of samples

1) Standard well: add 50 μL of standard with different concentrations into the wells of 96 well microplate.

   Sample well: add 50 μL of sample into the wells of 96 well microplate.

2) Add 50 μL of working solution into each well.

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

4) 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℃ for 30 min. Measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.		

▲Calculation

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:

$$\text{UA content (μmol/L)} = (\Delta F - b) \div a \times f$$

2. Tissue sample:

$$\text{UA content (μmol/ gprot)} = (\Delta F - b) \div a \times f \div C_{pr}$$

Note:

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: Absolute fluorescence intensity of sample ($F_{Sample} - F_{Blank}$)

f: The dilution factor of tested samples.

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

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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

▲Example analysis

Dilute 50 μL of human urine with reagent 1 for 100 times, take 50 μL of diluted sample and 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:

$$\text{UA content (μmol/L)} = \frac{(3077.9\text{-}277.3\text{-}141.88)}{227.73} \times 100 = 1167.49 \text{ μmol/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

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

▲ Plasma

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

▲ Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . 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℃ and then carry the assay.

▲ Tissue sample

Accurately weigh the tissue sample, add reagent 1 according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Note:

1. Homogenized medium: Reagent 1.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

▲ Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.