Uric Acid (UA) Colorimetric Assay

Catalog No: E-BC-K016-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 86 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.58 mg/L

Detection range: 0.58-100 mg/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 animal urine, serum, plasma samples.

▲Background

Uric acid, a purine metabolite, is degraded into allantoin by uric acid enzymes in most mammals. Due to the absence of uric acid 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

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	Specification	Storage
Reagent 1	1 g/L Uric Acid Standard	1 mL × 1 vial	2-8°C , 3 months
Reagent 2	Protein Precipitator	60 mL × 4 vials	2-8°C , 3 months
Reagent 3	Alkali Reagent	60 mL × 1 vial	2-8°C , 3 months
Reagent 4	Phosphotungstic Acid Reagent	60 mL × 1 vial	2-8°C , 3 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.

▲Materials prepared by users

Instruments

Spectrophotometer, Vortex mixer, Micropipettor, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (5 mL)

Reagents:

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

▲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.

▲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.

Pre-assay preparation

▲ 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 (0.58-100 mg/L).

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).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	690 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

▲Operating steps

The preparation of standard curve

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

The measurement of samples

(1) Standard tube: add 0.2 mL of diluted UA standard into a 5 mL EP tube (make duplicates for each concentration).
Sample tube: add 0.2 mL of sample into a 5 mL EP tube.

(2) Add 2 mL of reagent 2 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 mL of the supernatant, then add 0.5 mL of reagent 3 and 0.5 mL of reagent 4 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 $\triangle A_{690}=A_{Sample}-A_{Blank}$. (Note: The color stability of uric acid is poor, so it is suggested to finish the absorbance detection within 20 min, and the quantity of samples is better not over 20 for each batch.)

▲Operation table

	Standard tube	Sample tube
Standard of different concentrations (mL)	0.2	
Sample (mL)		0.2
Reagent 2 (mL)	2	2
Mix fully and stand for 10 min, then centrifuge at 1708 g for 5 min and take the supernatant for the detection.		
Supernatant (mL)	1.6	1.6
Reagent 3 (mL)	0.5	0.5
Reagent 4 (mL)	0.5	0.5
Mix fully and stand for 10 min at room temperature. Set the spectrophotometer to zero with double distilled water and measure the OD value (A) of each tube at 690 nm with 1 cm optical path cuvette. Calculate $\triangle A_{690}=A_{Sample}-A_{Blank}$.		

▲Calculation

Plot the standard curve by using OD value 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 OD value of sample.

The standard curve is: y= ax + b.

$$\text{Uric acid content (mg/L)}=(\Delta A_{690}-b)\div a\times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{690}: $OD_{Sample} - OD_{Blank}$.

▲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 3 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	0.58-100 mg/L	Average intra-assay CV (%)	1.8
Sensitivity	0.58 mg/L	Average inter-assay CV (%)	2.6
Average recovery rate (%)	105		

▲Example analysis

Take 0.2 mL of human serum, carry the assay according to the operation table.

The results are as follows:

standard curve: y= 0.0028x - 0.0112 (R^2=0.994), the average OD value of the sample well is 0.164, and the calculation result is:

Uric acid content (mg/L)= (0.164+0.0112)÷0.0028=62.571 mg/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. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲ 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. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲ Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲ Notes 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.