Technical Manual

Uric Acid (UA) Colorimetric Assay Kit

- **Catalogue Code: MAES0029**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

1.30-80 mg/L

Sensitivity:

1.30 mg/L

Storage:

2-8°C for 3 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 3 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. Detection Principle

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. Uric acid content can be calculated by measuring the OD value at 690 nm.

5. Kit Components & Storage

Item	Specification	Storage
Uric Acid Standard (1 g/L)	1 mL × 1 vial	2-8°C, 3 months
Protein Precipitator	30 mL × 1 vial	2-8°C, 3 months
Alkali Reagent	6 mL × 1 vial	2-8°C, 3 months
Phosphotungstic Acid Reagent	6 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (680-700 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

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.

7. Reagent Preparation:

Bring all reagents to room temperature before use.

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 on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is recommended), 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 on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Urine:
Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the urine 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 (1.30-80 mg/L).

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);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 690 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 1 g/L uric acid standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 50, 60, 80 mg/L.

The measurement of samples

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: The color stability of uric acid is poor, so it is suggested to finish the absorbance detection within 20 min.)

Operation Table

	Standard tube	Sample tube
Standard with different concentrations (μL)	25	
Sample (μL)		25
Protein precipitator (μL)	250	250
Mix fully with the vortex mixer, stand the tubes for 5 min. Centrifuge at 2000 g for 5 min, take 160 μL of the supernatant to the microplate.		
Supernatant (μL)	160	160
Alkali reagent (μL)	50	50
Phosphotungstic acid reagent (μL)	50	50
Mix fully with microplate reader for 10 s and stand at room temperature for 15 min. Measure the OD value of each well at 690 nm with microplate reader.		

11. Calculations

Plot the standard curve by using fluorescence 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 fluorescence value of sample. The standard curve is: y= ax + b.

Serum (plasma) and other liquid sample:

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

y: ODstandard – ODBlank.

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{690}: Absolute OD (OD_{Sample} – OD_{Blank})

f: the dilution multiple of tested samples.

12. Performance Characteristics

Detection Range	1.30-80 mg/L
Sensitivity	1.30 mg/L
Average recovery rate (%)	96
Average inter-assay CV (%)	4.0
Average intra-assay CV (%)	2.0

Analysis

Take 25 μL of human serum, 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 well is 0.098, the average OD value of the blank well is 0.037, and the calculation result is:

$$\textbf{UA content (mg/L)} = (0.098 - 0.037 + 0.00366) \div 0.00108$$

$$= 59.87 \text{ mg/L}$$

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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Contact Details

Email: info@assaygenie.com

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