TECHNICAL MANUAL

Uric Acid (UA) Colorimetric Assay Kit

- **SKU CODE:** MAES0029
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Protein precipitation and centrifugation
- Add alkali reagent and phosphotungstic acid reagent
- Measure absorbance at 690 nm
- ![img-0.jpeg](img-0.jpeg)

2. Intended use

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

3. Detection principle

Uric acid (UA) in the protein-free filtrate reduces phosphotungstic acid to form tungsten blue, allantoin, and carbon dioxide. The intensity of the blue color is proportional to the uric acid concentration. The uric acid content can be calculated by measuring the absorbance at 690 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
Reagent 1	1 g/L Uric Acid Standard	1 mL × 1 vial	1 mL × 1 vial	5 mL × 1 vial	2-8 °C, 12 months
Reagent 2	Protein Precipitator	15 mL × 1 vial	30 mL × 1 vial	50 mL × 3 vials	2-8 °C, 12 months
Reagent 3	Alkali Reagent	3 mL × 1 vial	6 mL × 1 vial	30 mL × 1 vial	2-8 °C, 12 months
Reagent 4	Phosphotungstic Acid Reagent	3 mL × 1 vial	6 mL × 1 vial	30 mL × 1 vial	2-8 °C, 12 months,

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3(500 Assays)	Storage
					protected from light
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (680-700 nm, optimum wavelength: 690 nm), Micropipettor, Centrifuge, Vortex mixer

Reagents:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of Standard Curve Dilution::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1 g/L Uric Acid Standard solution with deionized water to serial concentrations. The recommended dilution gradient is as follows: 0, 10, 20, 30, 40, 50, 60, 80 mg/L. Reference is as follows: Item: ①, ②, ③, ④, ⑤, ⑥, ⑦, ⑧ Concentration (mg/L): 0, 10, 20, 30, 40, 50, 60, 80 1g/L Uric Acid Standard (μL): 0, 10, 20, 30, 40, 50, 60, 80 Deionized water (μL): 1000, 990, 980, 970, 960, 950, 940, 920

7. Sample preparation

1. **Sample preparation:** Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for a month. Urine: Collect fresh urine and centrifuge at 10,000 × g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection. If not detected on the same day, the urine can be stored at -80 °C for a month.
2. **Dilution of sample:** 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). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The supernatant after centrifugation must be clarified.

2. Tungsten blue shows poor color stability; it is recommended to complete the colorimetric measurement within 20 minutes after color development.

9. Operating steps

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 thoroughly with the vortex mixer.

3. Let the tubes stand for 5 minutes. Centrifuge at 2,000 × g for 5 minutes (The supernatant should be clarified).

4. Take 160 μL of the supernatant to the corresponding wells of the microplate.

5. Add 50 μL of alkali reagent and 50 μL of phosphotungstic acid reagent in order. Mix thoroughly with microplate reader for 10 seconds and incubate at room temperature for 15 minutes.

6. Measure the absorbance of each well at 690 nm with the microplate reader. (Note: Tungsten blue shows poor color stability; it is recommended to complete the colorimetric measurement within 20 minutes after color development).

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean absorbance value of the blank (Standard #①) from all standard readings. This is the corrected absorbance value.

3. Plot the standard curve using the corrected absorbance value of standards and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

UA content (mg/L) = (ΔA_690 - b) ÷ a × f

[Note]

ΔA_690: Corrected absorbance value, A_{Sample} - A_{Blank}

f: Dilution factor of sample before test.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	2.40	35.00	60.00
%CV	2.3	1.8	1.9

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	0.10	0.40	0.76
%CV	4.4	2.1	0.7

Recovery

Three samples of high concentration, middle concentration, and low concentration were tested with 6 replicates each to obtain an average recovery rate of 96%.

Recovery

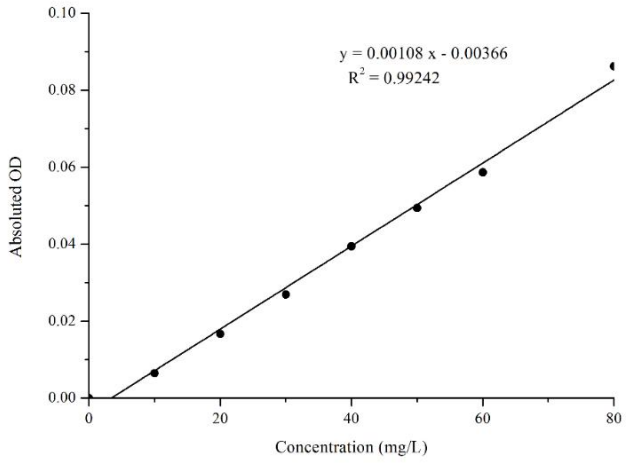
Standard 1	Standard 2	Standard 3
15	35	55
14.4	34.3	51.7
96	98	94

Sensitivity

The analytical sensitivity of the assay is 1.30 mg/L UA. This was determined by adding two standard deviations to the mean absorbance obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the absorbance values of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (mg/L)	Average OD	Corrected OD
0	0.037	0
10	0.043	0.006

Concentration (mg/L)	Average OD	Corrected OD
20	0.053	0.017
30	0.064	0.027
40	0.076	0.039
50	0.086	0.049
60	0.095	0.059
80	0.123	0.086

12. Appendix II Example Analysis

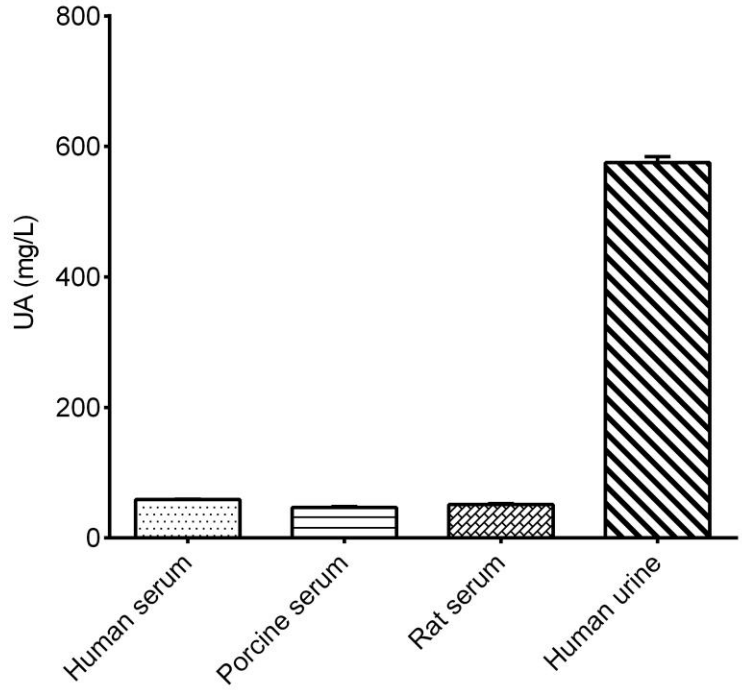
Example analysis:

For human serum, take 25 µL of human serum and perform the assay according to the operating procedures. The results are as follows:

Standard curve: y = 0.00108x - 0.00366, the average absorbance value of the sample is 0.098, the average absorbance value of the blank is 0.037, and the calculation result is:

UA content (mg/L) = (0.098 - 0.037 + 0.00366) ÷ 0.00108 = 59.87 mg/L

Detect human serum, porcine serum, rat serum, and human urine (diluted 10-fold) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.