TECHNICAL MANUAL

Uric Acid (UA) Fluorometric Assay Kit

- **SKU CODE:** MAES0005
- **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
- Measure fluorescence value

2. Intended use

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

3. 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 a non-fluorescent probe into a fluorescent substance. By measuring the fluorescence value of the system, the corresponding uric acid content can be calculated.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	60 mL x 1 vial	60 mL x 2 vials	-20°C, 12 months
Reagent 2	Probe Solution	0.12 mL x 1 vial	0.24 mL x 1 vial	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent 1	0.12 mL x 1 vial	0.24 mL x 1 vial	-20°C, 12 months
Reagent 4	Enzyme Reagent 2	0.6 mL x 1 vial	1.2 mL x 1 vial	-20°C, 12 months
Reagent 5	20 µmol/L Uric Acid Standard	1.5 mL x 1 vial	1.5 mL x 1 vial	-20°C, 12 months
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), micropipettor, vortex mixer, centrifuge

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of working solution:** For each well, prepare 50 µL of working solution (mix well 36 µL of buffer solution, 2 µL of probe solution, 2 µL of enzyme reagent 1, and 10 µL of enzyme reagent 2). The working solution should be prepared immediately before use and protected from light.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 20 µmol/L uric acid standard with buffer solution to serial concentrations. The recommended dilution gradient is as follows: 0, 2, 4, 6, 8, 10, 12, 15 µmol/L.

7. Sample preparation

Sample preparation:

Serum, plasma, and other liquid samples: Detect directly. If not detected on the same day, serum or plasma can be stored at -80°C for one month.

Tissue samples:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL buffer solution with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000 × g for 10 min to remove insoluble material. Collect supernatant and keep on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant.

Dilution of samples:

The recommended dilution factors for different samples are as follows (for reference only):

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 buffer solution. For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. Operating steps

1. Standard wells: Add 50 µL of standards with different concentrations into the wells. Sample wells: Add 50 µL of sample into the wells.
2. Add 50 µL of working solution into each well.
3. Mix thoroughly with microplate reader for 5 seconds and incubate at 37°C for 30 minutes.
4. Measure the fluorescence value at excitation wavelength of 535 nm and emission wavelength of 587 nm.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using absolute fluorescence values of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum (plasma) and other liquid samples:

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

2. Tissue samples:

UA content (µmol/g_{prot}) = (ΔF − b) ÷ a × f ÷ C_{pr}

[Note]

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, g_{prot}/L

10. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

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

Inter-assay Precision

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

Recovery

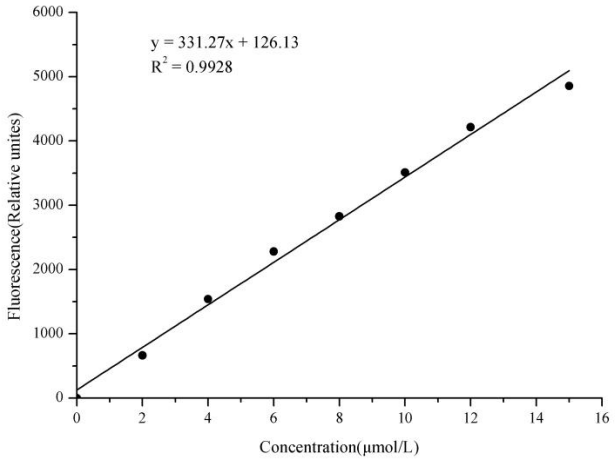
Three samples of high, middle, and low concentrations were tested with each concentration analyzed 6 times in parallel to achieve an average recovery rate of 101%.

Sensitivity

The analytical sensitivity of the assay is 0.03 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

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



11. Appendix II Example Analysis

Example analysis:

Dilute 50 μL of human urine with buffer solution 100 times, take 50 μL of diluted sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 227.73x + 141.88

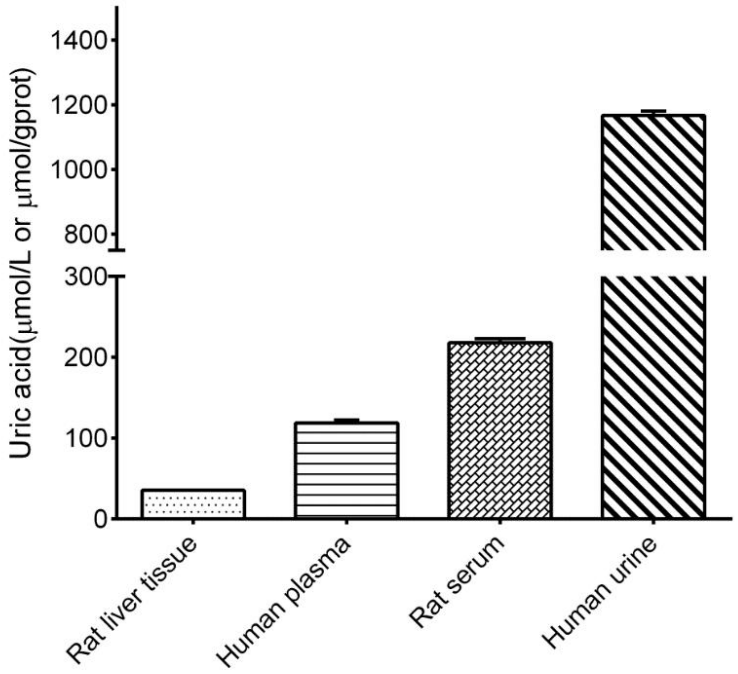
Average fluorescence value of sample: 3077.9

Average fluorescence value of blank: 277.3

Calculation result:

UA content (μmol/L) = (3077.9 - 277.3 - 141.88) ÷ 227.73 × 100 = 1167.49 μmol/L

Detect 10% rat liver tissue homogenate (protein concentration is 6.94 g_{prot}/L, diluted 20 times), human plasma (diluted 10 times), rat serum (diluted 20 times), and human urine (diluted 100 times) according to the protocol. The results are as follows:



12. 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 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 pretest 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.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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