TECHNICAL MANUAL

Uricase Acitivity Colorimetric Assay Kit

- **SKU CODE:** MAES0529
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add samples and reagents
- Incubate and measure absorbance
- Calculate uricase activity

2. Intended use

This kit can measure uricase activity in animal and plant tissue samples.

3. Detection principle

Uricase is an enzyme in the purine decomposition pathway that specifically catalyzes the oxidation of uric acid into allantoin, which has higher solubility, thereby reducing blood uric acid levels.

The detection principle of this kit is based on uricase oxidizing its substrate, which has a maximum absorption peak at 293 nm. Uricase activity is calculated by measuring the reduction in absorbance at 293 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	35 mL x 1 vial	35 mL x 2 vials	-20 °C, 12 months, protect from light
Reagent 2	Substrate	Powder x 4 vials	Powder x 8 vials	-20 °C, 12 months, protect from light
Reagent 3	Matrix Solution	6 mL x 1 vial	12 mL x 1 vial	-20 °C, 12 months, protect from light
	UV-Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (optimum wavelength: 293 nm), incubator, ultrasonic cleaner

Reagents:

PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Allow all reagents to equilibrate to 25 °C before use.
2. **Substrate working solution preparation:** Dissolve one vial of substrate with 1 mL of matrix solution. Mix well using an ultrasonic cleaner to dissolve completely. Store at -20 °C for up to 3 days.
3. **Working solution preparation:** Before testing, prepare sufficient working solution. For example, to prepare 150 µL of working solution, mix 5 µL of substrate working solution with 145 µL of buffer solution. The working solution should be prepared fresh and used within 8 hours.
4. **Reaction working solution preparation:** For each well, prepare 160 µL of reaction working solution by mixing 32 µL of working solution with 128 µL of buffer solution. The reaction working solution should be prepared fresh and used within 8 hours.

7. Sample preparation

1. **Tissue sample preparation:** 1. Harvest the required amount of tissue for each assay (initially recommended: 20 mg). 2. Wash tissue in cold PBS (0.01 M, pH 7.4). 3. Homogenize 20 mg tissue in 180 µL buffer solution using a Dounce homogenizer at 4 °C. 4. Centrifuge at 10,000 xg for 10 min at 4 °C to remove insoluble material. Collect the supernatant and keep on ice for detection. Use the prepared tissue supernatant within 4 hours.
2. **Sample dilution:** The recommended dilution factors for different samples are as follows (for reference only): - 10% Mouse liver tissue homogenate: 1-3 - 10% Porcine liver tissue homogenate: 1-3 - 10% Spinach tissue homogenate: 1 Note: Use buffer solution as diluent. For dilution of other sample types, perform a pretest to confirm the appropriate dilution factor.

8. Operating steps

1. Add reagents to wells: Blank well: Add 20 µL of buffer solution Sample well: Add 20 µL of sample Control well: Add 20 µL of sample

2. Add 160 µL of reaction working solution to blank wells and sample wells. Add 160 µL of buffer solution to control wells.
3. Mix thoroughly using the microplate reader for 5 seconds and measure the absorbance of each well at 293 nm (record as A1).
4. Incubate at 37 °C for 20 minutes, protected from light.
5. Mix thoroughly using the microplate reader for 5 seconds and measure the absorbance of each well at 293 nm (record as A2).

9. Calculation

Tissue sample:

Definition: One unit is defined as the amount of enzyme in 1 kg tissue that consumes 1 µmol of uric acid per minute in a 1-liter reaction system at 37 °C.

Uricase activity (U/kg wet weight) = $(deltaA_{sample} - deltaA_{control} - deltaA_{blank}) \times V_{total} \times f \div \varepsilon \div d \div V_{sample} \div T \div m \div V \times 10^6$

Where:

$deltaA_{sample}$: The absorbance change of sample wells ($A_1 - A_2$)

$deltaA_{control}$: The absorbance change of control wells ($A_1 - A_2$)

$deltaA_{blank}$: The absorbance change of blank wells ($A_1 - A_2$)

f: Dilution factor of sample before test

V_{total}: Volume of reaction system, 0.18 mL

V_{sample}: Volume of sample added to reaction system, 0.02 mL

T: Reaction time, 20 min

ε: Molar extinction coefficient, 12,500 $L \bullet mol^{-1} \bullet cm^{-1}$

d: Optical path length, 0.6 cm

m: Wet weight of sample, g

V: Volume of buffer solution in preparation step, mL

10^6: Conversion factor (1 mol/L = 10^6 µmol/L)

10. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

Three mouse liver 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 (U/L)	0.20	0.40	0.80
%CV	2.6	3.4	3.1

Inter-assay Precision

Three mouse liver 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 (U/L)	0.20	0.40	0.80
%CV	6.7	8.8	8.1

Recovery

Three samples of high, medium, and low concentrations were tested with 6 parallel measurements each to obtain an average recovery rate of 99%.

Recovery

Parameters	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.20	0.40	0.80
Observed Conc. (U/L)	0.20	0.40	0.80
Recovery rate (%)	99.0	100.0	99.5

Sensitivity

The analytical sensitivity of the assay is 0.06 U/L. 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.

11. Appendix II Example Analysis

Example analysis:

Take 20 µL of 10% mouse liver tissue homogenate diluted 2-fold and perform the assay according to the operating steps. The results are as follows:

The average A_1 of the blank well is 0.790, the average A_1 of the sample well is 1.465, the average A_1 of the control well is 0.742. The average A_2 of the blank well is 0.787, the average A_2 of the sample well is 1.395, the average A_2 of the control well is 0.716.

$deltaA_{sample}$ = 1.465 - 1.395 = 0.070

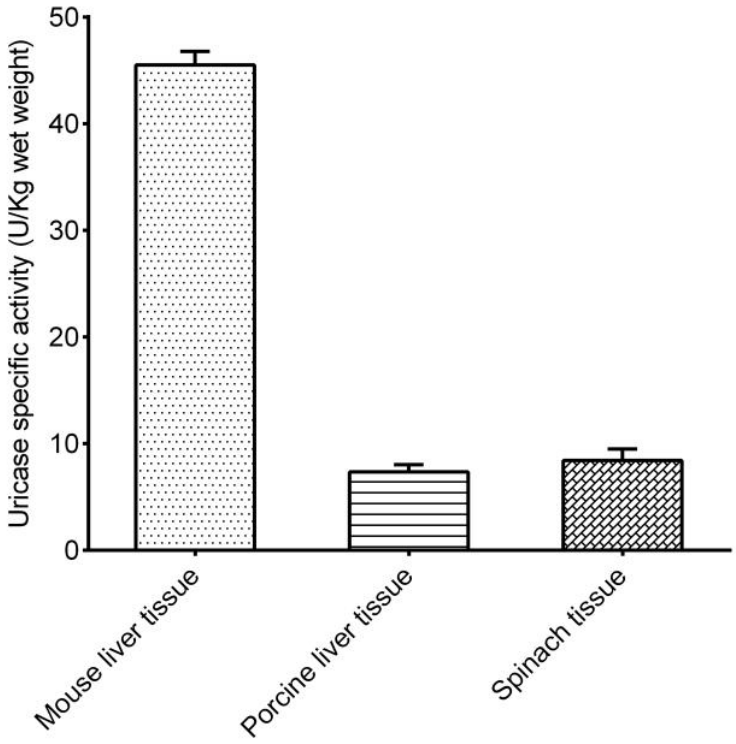
$deltaA_{control}$ = 0.742 - 0.716 = 0.026

$deltaA_{blank}$ = 0.790 - 0.787 = 0.003

Calculation result:

Uricase activity (U/kg wet weight) = (0.07 - 0.026 - 0.003) × 0.18 × 2 ÷ 12500 ÷ 0.6 ÷ 0.02 ÷ 20 ÷ 0.1 × 0.9 × 1 × 10^6 = 44.28 U/kg wet weight

Detection of 10% mouse liver tissue homogenate (diluted 2-fold), 10% porcine liver tissue homogenate, and 10% spinach tissue homogenate according to the protocol yielded the following results:



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 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.