3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Uric Acid Assay Kit

Catalog Number **MAK077**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

In humans and primates, uric acid is the end product of purine metabolism. It is produced by the oxidation of xanthine and hypoxanthine by xanthine oxidase and excreted in urine. High serum levels of uric acid, hyperuricemia, are associated with insulin resistance, cardiovascular disease, and gout. The mechanisms leading to hyperuricemia are typically either increased uric acid production or decreased urine excretion. Increased serum uric acid may be a marker of renal disease.[1]

In this assay, Uric Acid concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/ λ_{em} = 587 nm) product, proportional to the uric acid present.

Components

The kit is sufficient for 100 assays in 96 well plates.

Uric Assay Buffer Catalog Number MAK077A	25 mL
Uric Acid Probe, in DMSO Catalog Number MAK077B	1 vl
Uric Acid Enzyme Mix Catalog Number MAK077D	1 vl
Uric Acid Standard, 2 nmole/µL Catalog Number MAK077E	1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Uric Acid Assay Buffer – Allow buffer to come to room temperature before use.

Uric Acid Probe – Thaw at room temperature to melt solution prior to use. Aliquot and store protected from light and moisture at –20 °C.

Uric Acid Enzyme Mix – Reconstitute with 220 µL of Uric Acid Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Uric Acid Standards for Colorimetric Detection
Add 0, 4, 8, 12, 16, and 20 µL of the Uric Acid Standard (2 nmole/µL) into a 96 well plate, generating 0 (blank), 8, 16, 24, 32, and 40 nmole/well standards. Add Uric Acid Assay Buffer to each well to bring the volume to 50 µL.

Uric Acid Standards for Fluorometric Detection
Dilute 20 µL of the Uric Acid Standard (2 nmole/µL) with 180 µL of Uric Acid Assay Buffer to prepare a 0.2 nmole/µL standard solution. Add 0, 4, 8, 12, 16, and 20 µL of the Uric Acid standard solution into a 96 well plate, generating 0 (blank), 0.8, 1.6, 2.4, 3.2, and 4.0 nmole/well standards. Add Uric Acid Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10 mg) or cells (2×10^6) should be rapidly homogenized in 4 volumes of cold Uric Acid Assay buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material.

If using serum samples, add 2-20 µL of serum to wells (normal serum contains ~0.3 nmol/µL uric acid) and bring samples to a final volume of 50 µL with Uric Acid Assay Buffer.

If using urine samples, urine can be assayed directly without dilution as long as the readings fall within standard curve range.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Volume**
Uric Acid Assay Buffer	46 µL
Uric Acid Probe	2 µL
Uric Acid Enzyme Mix	2 µL

2. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 590 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Uric Acid Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Uric Acid standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Uric Acid

$$S_a/S_v = C$$

S_a = Amount of Uric Acid in unknown sample (nmole) from standard curve

S_v = Sample volume (µL) added into the wells

C = Concentration of Uric Acid in sample

Uric Acid molecular weight: 168.1 g/mole

Sample Calculation

Amount of Uric Acid (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 µL

Concentration of Uric Acid in sample

$$5.84 \text{ nmole}/50 \text{ µL} = 0.1168 \text{ nmole/µL}$$

$$0.1168 \text{ nmole/µL} \times 168.1 \text{ ng/nmole} = 19.63 \text{ ng/µL}$$

Reference

1. Kang, D.-H., A Role for Uric Acid in the Progression of Renal Disease. *Journal of the American Society of Nephrology*, **13(12)**, 2888–2897 (2002).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of aged kit or improperly stored reagents	Check the receipt date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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