Technical Bulletin

Uric Acid Assay Kit

Catalogue Number MAK630

Product Description

Uric acid is the breakdown product of purines such as adenosine and inosine. Humans and other primates are incapable of further metabolizing uric acid. In humans, uric acid is excreted via the kidneys, but a failure to remove excess uric acid can lead to conditions such as gout or uric acid kidney stones.

In this assay, uric acid is enzymatically converted to allantoin, releasing H_2O_2. The resulting H_2O_2 reacts with a specific dye to form a pink-colored product. The change in OD570nm or fluorescence intensity at λex/em = 530/585nm is directly proportional to the uric acid present in the sample.

Linear detection range:
Colorimetric assay 15 - 1000 µM Uric Acid.
Fluorometric assay 4 - 300 µM Uric Acid.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK630A
- Standard (1 mM) 400 µL
  Catalogue Number MAK630B
- HRP Enzyme 100 µL
  Catalogue Number MAK630C
- Dye Reagent 120 µL
  Catalogue Number MAK630D
- UO Enzyme 100 µL
  Catalogue Number MAK630E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Plate reader capable of λex/em = 530/585 nm or OD 570nm.
- Clear plates for colorimetric assays (Catalogue number M2936 or equivalent).

  Black plates with clear bottoms for fluorescence assays (Catalogue number CLS3631 or equivalent).

  Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes

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.

Storage/Stability

The kit is shipped on dry ice. Store at -20°C upon receipt.

Preparation Instructions

Colorimetric Assay Sample Preparation

Serum, urine and cell culture media samples must be diluted 10, 10 and 2-5 fold respectively with Assay Buffer or 100 mM Tris-HCl (pH 7.5) prior to analysis.

All samples need to be clear and debris-free.

Note: SH-containing reagents (e.g. β-mercaptoethanol, dithiothreitol, > 5 µM),

sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere in this assay and should be avoided in sample preparation.

Fluorometric Assay Sample Preparation

Serum and plasma, cell culture media, and cell lysates should be diluted 1:50 to 1:100 with Assay Buffer or 100 mM Tris-HCl (pH 7.5).

Urine is not recommended for the Fluorometric assay.

Colorimetric Standard Preparation

Prepare standards as shown in Table 1 in separate wells of a clear 96-well plate.

Table 1.
Colorimetric Standard Preparation

Std #	1 mM Standard (µL)	Water (µL)	Conc. (µM)
1	10	0	1000
2	6	4	600
3	3	7	300
4	0	10	0

Fluorometric Standard Preparation

Prepare standards for the fluorometric assay as seen below in Table 2. Transfer 10 µL of each standard into a black clear-bottom 96-well plate.

Table 2.
Fluorometric Standard Preparation

Std #	1 mM Standard (µL)	Water (µL)	Conc. (µM)
1	30	70	300
2	15	85	150
3	5	95	50
4	0	100	0

Procedure

Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay

Assay Reaction

1. Transfer 10 µL of each sample into separate wells of the plate.
2. Prepare enough Working Reagent by mixing, for each well, 95 µL Assay Buffer, 1 µL UO enzyme, 1 µL HRP Enzyme and 1 µL Dye Reagent.
3. Add 90 µL Working Reagent to each well. Immediately tap plate to mix.
4. Colorimetric: Read optical density at 570 nm in kinetic mode for 30 minutes.

   Fluorometric: Read fluorescence (λex/em = 530/585 nm) for 10 minutes.

Results

Calculations

Subtract Blank value (Standard #4) from the standard values and plot the ΔOD or ΔF against standard concentrations.

Use a hyperbolic equation to fit the standard curve and compute the Uric Acid concentration of Sample.

Note: If the resulting Uric Acid concentration of a sample is higher than 1000 µM in the Colorimetric Assay or 300 µM in the Fluorometric Assay, dilute sample in dH_2O and repeat the assay. Multiply result by the dilution factor, *n*.

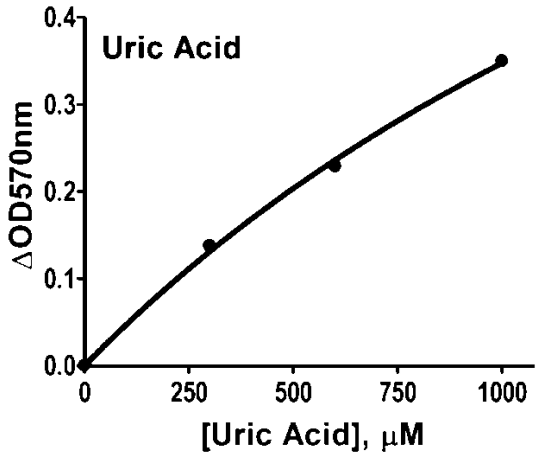

Figure 1.
Exemplary colorimetric standard curve

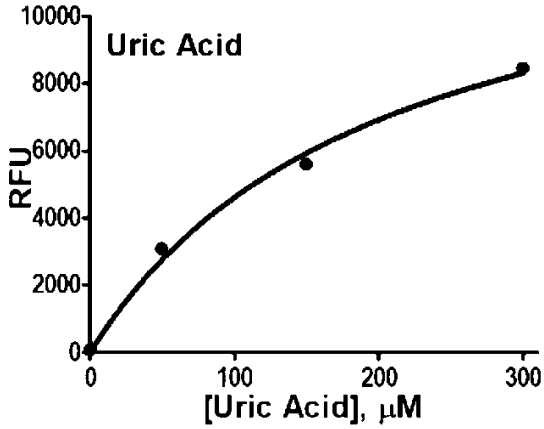

Figure 2.
Exemplary fluorometric standard curve

References

1. Borghi C et al (2022) Uric Acid and Hypertension: a Review of Evidence and Future Perspectives for the Management of Cardiovascular Risk. Hypertension. 79:1927–1936.
2. Jin M et al (2012) Uric Acid, Hyperuricemia and Vascular Diseases. Front Biosci. 17: 656–669.
3. Roman YM (2023) The role of uric acid in human health: Insights from the Uricase gene. J Pers Med. 13(9):140.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak630pis Rev 02/26