Technical Bulletin

Uricase Assay Kit

Catalogue Number MAK631

Product Description

Uricase (urate oxidase; EC 1.7.3.3) catalyzes oxidation of uric acid to allantoin, releasing hydrogen peroxide. Uricase activity is found in plants, animals, and bacteria, but absent in hominids; elevated uric acid in humans is associated with gout.

The Uricase Assay Kit provides a rapid fluorometric method to quantify uricase activity. 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 fluorescence intensity at 530/585 nm is directly proportional to the uricase activity in the sample. The linear detection range is 0.3 - 100 U/L Uricase.

Components

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

- Assay Buffer 5 mL
  Catalogue Number MAK631A
- Substrate 5 mL
  Catalogue Number MAK631B
- HRP Enzyme 120 µL
  Catalogue Number MAK631C
- Standard (3% H_2O_2) 100 µL
  Catalogue Number MAK631D
- Dye Reagent 120 µL
  Catalogue Number MAK631E
- Uricase Control 20 µL
  Catalogue Number MAK631F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor).
- Fluorescent multiwell plate reader capable of reading at $\lambda_{Ex}/\lambda_{Em}$ = 530/585 nm.
- 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 components at -25°C to -10°C upon receipt.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use. Keep thawed enzyme-containing tubes on ice during assay.

Sample Preparation

Liquid samples such as serum and plasma can be assayed directly.

Tissue and cell lysates 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.

Preparation of H_2O_2 Standards

1. Prepare a 10 mM H_2O_2 stock by mixing 10 µL of 3% H_2O_2 with 870 µL of purified water (final, 10 mM).

2. Prepare a 100 µM H_2O_2 working solution by diluting 10 µL of the 10 mM stock with 990 µL of purified water.

3. Further dilute standards as in Table 1.

Table 1.
Standard Preparation

Std #	100 mM Standard (µL)	Water (µL)	Conc. (µM)
1	100	0	100
2	60	40	60
3	30	70	30
4	0	100	0

Procedure

All samples and standards should be run in technical duplicates or triplicates.

Assay Reaction

1. Transfer 10 µL standards and samples into separate wells of a black flat-bottom 96-well plate.
2. Dilute the Uricase Control 1:100 in Assay Buffer then transfer 10 µL of the enzyme to the Control well.
3. Prepare enough Working Reagent by mixing, for each well, 50 µL Assay Buffer, 50 µL 1 mM Uric Acid Substrate, 1 µL HRP enzyme and 1 µL Dye Reagent.
4. Add 90 µL Working Reagent to each well.
5. Read fluorescence intensity at λex = 530 nm and λ em = 585 nm in kinetic mode for 10 minutes.

   Note: If the observed uricase activity is higher than 100 U/L, dilute sample in Assay Buffer and repeat the assay. Multiply result by the dilution factor *n*.

Results

Calculations

Subtract blank F_{10} (water, #4) from all standard F_{10} values and plot the ΔF against standard concentrations.

Determine the slope using linear regression.

Calculate the ΔF_{Sample} of all samples by subtracting F_0 from F_{10} for each sample. Do the same for the blank (water, standard #4) to get ΔF_{Blank}.

Calculate the activity using the equation below:

$$\text{Uricase Activity} = \frac{\Delta F_{SAMPLE} - \Delta F_{BLANK}}{\text{Slope } (\mu M^{-1}) \bullet t} \times n \text{ (U/L)}$$

Where:

ΔF_{Sample} and ΔF_{Blank} are the changes in fluorescence values of the sample and blank, respectively.

Slope is the slope of the H_2O_2 standard curve

t is the incubation time (10 minutes)

n is the dilution factor

Unit definition: 1 U Uricase will generate 1 µmol of H_2O_2 from Uric Acid per minute at 25 °C at pH 7.5.

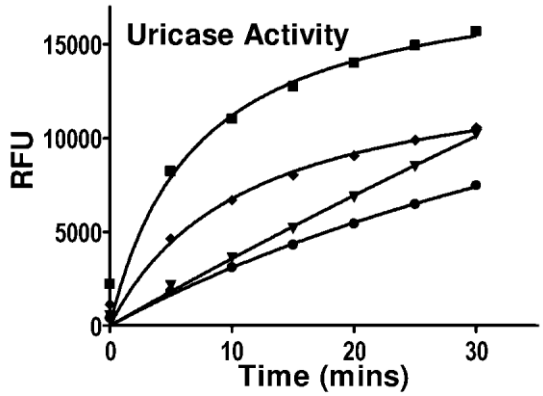

Figure 1.
Fluorometric Assay. ■100 U/L Uricase, ♦ 60 U/L Uricase, ● Rat Liver Microsomes, ▼ Pea Roots.

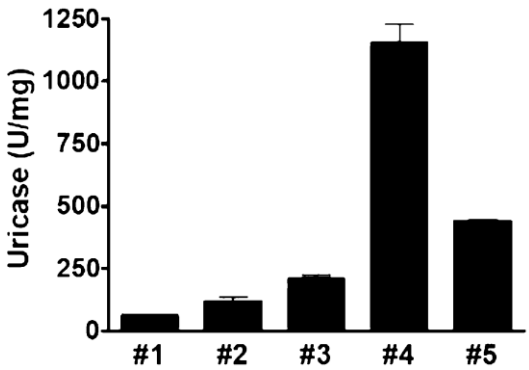

Figure 2.
Uricase Specific Activity in rat liver cytosol (#1), rat liver S9 fraction (#2), rat liver microsomes (#3), pea roots (#4) and pea shoots (#5)

References

1. Kratzer, JT., et al., (2014) Evolutionary history and metabolic insights of ancient mammalian uricases. Proc Natl Acad Sci USA 111(10):3763-8.
2. Maiuolo J et al (2016) Regulation of uric acid metabolism and excretion. Int J Cardiol. 213:8-14
3. Roman YM (2023) The role of uric acid in human health: Insights from the Uricase gene. J Pers Med. 13(9):1409

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