EnzyFluo™ Uricase Assay Kit (EURIC-100)

Quantitative Fluorimetric Uricase Activity Determination

DESCRIPTION

URICASE or *URATE OXIDASE (E.C. 1.7.3.3)* initiates the oxidation of uric acid, eventually yielding allantoin. Uricase activity is found throughout plants, animals, and bacteria, but it is not found in hominids. As a result, humans develop gout when the levels of uric acid become too high. BioAssay Systems' method provides a simple and high-throughput assay for measuring uricase enzyme 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.

KEY FEATURES

Sensitive. Use 10 µL samples. Linear detection range: fluorimetric assay 0.3 - 100 U/L Uricase.

Fast. Run time is 10 minutes for rapid results.

Convenient. Room temperature "mix-and-read" procedure can be readily automated for high-throughput assay of thousands of samples per day.

APPLICATIONS

Assays: Uricase activity in biological samples such as cell culture, non-human biofluids, cell extracts, and plant tissue extracts.

KIT CONTENTS

Assay Buffer: 5 mL
Substrate: 5 mL 1mM Uric Acid
Uricase Control: 20 µL
Dye Reagent: 120 µL
HRP Enzyme: 120 µL
Standard: 100 µL 3% H_2O_2

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

FLUORIMETRIC ASSAY

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.

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.
2. *H_2O_2 Standard Curve.* Mix 10 µL 3% H_2O_2 and 870 µL dH_2O (final 10 mM) then mix 10 µL of the 10 mM H_2O_2 with 990 µL dH_2O to yield 100 µM H_2O_2. Prepare standards as shown in the Table below.

No	100 µM H_2O_2 + H_2O	Vol (µL)	H_2O_2 (µM)
1	100 µL + 0 µL	100	100
2	60 µL + 40 µL	100	60
3	30 µL + 70 µL	100	30
4	0 µL + 100 µL	100	0

Transfer 10 µL standards and samples into separate wells of a black flat-bottom 96-well plate. Dilute the Uricase Control 1:100 in Assay Buffer then transfer 10 µL of the enzyme to the Control well.

3. *Fluorescence reaction.* 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. Add 90 µL Working Reagent to each well.
4. Read fluorescence intensity at λ_{ex} = 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.

CALCULATION

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:

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

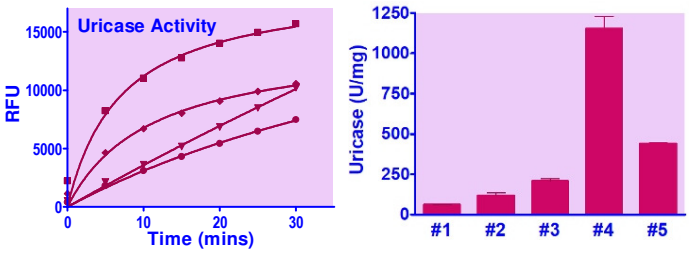
$$= \frac{\Delta F_{SAMPLE} - \Delta F_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \text{ (U/L)}$$

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), and *n* is the dilution factor. Note: in the standard protocol, the reaction volume/sample volume value cancels out the time value.

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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Uricase-containing samples, pipetting devices, centrifuge tubes, black flat-bottom uncoated 96-well plates, and fluorescence plate reader.



Left**:* 96-Well Fluorimetric Assay. ■ 100U/L Uricase, ♦ 60 U/L Uricase, ● Rat Liver Microsomes, ▼ Pea Roots. ***Right: Uricase Specific Activity in rat liver cytosol (#1), rat liver S9 fraction (#2), rat liver microsomes (#3), pea roots (#4) and pea shoots (#5)

LITERATURE

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