EnzyChrom™ Uric Acid Assay Kit II (EUAC-100)

Quantitative Colorimetric/Fluorimetric Uric Acid Determination

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.

BioAssay Systems' method provides a simple and high-throughput assay for measuring uric acid levels. 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 $\lambda_{ex/em}$ = 530/585nm is directly proportional to the uric acid present in the sample.

KEY FEATURES

Sensitive. Use 10 µL samples. Linear detection range: Colorimetric assay 15 – 1000 µM Uric Acid. Fluorimetric assay 4 – 300 µM Uric Acid.

Fast. Run time is 30 minutes for Optical Density method and 10 minutes for Fluorimetric method 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

Uric acid determination in biological samples such as cell culture media, biofluids (serum, urine), cell and tissue extracts.

KIT CONTENTS

Assay Buffer: 10 mL
Standard: 400 µL 1mM
UO Enzyme: 100 µL
Dye Reagent: 120 µL
HRP Enzyme: 100 µL

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.

COLORIMETRIC ASSAY

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.

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.
2. *Uric Acid Standard Curve.* Prepare standards as shown in the Table below in separate wells of a clear 96-well plate.

No	1mM Uric Acid + H_2O	Vol (µL)	Uric Acid (µM)
1	10 µL + 0 µL	10	1000
2	6 µL + 4 µL	10	600
3	3 µL + 7 µL	10	300
4	0 µL + 10 µL	10	0

Transfer 10 µL samples into separate wells of the plate.

3. *Colorimetric reaction.* 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. Add 90 µL Working Reagent to each well. Immediately tap plate to mix.
4. Read Optical Density at 570 nm in kinetic mode for 30 minutes.

FLUORIMETRIC ASSAY

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 Fluorimetric assay. The assay procedure is the same as with the Colorimetric Assay, except that the standards below (10 µL per well) should be used and reading fluorescence at $\lambda_{em/ex}$ = 585/530nm for 10min.

No	1mM Uric Acid + H_2O	Vol (µL)	Uric Acid (µM)
1	30 µL + 70 µL	100	300
2	15 µL + 85 µL	100	150
3	5 µL + 95 µL	100	50
4	0 µL + 100 µL	100	0

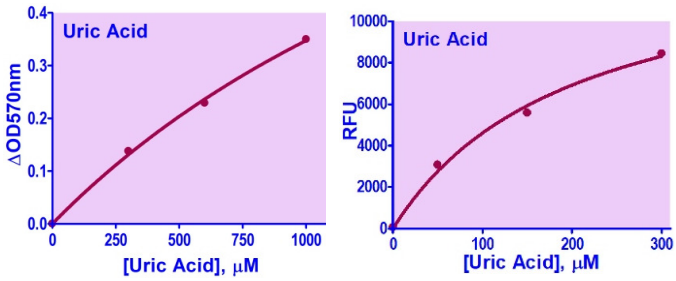
CALCULATION

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 Fluorimetric Assay, dilute sample in dH_2O and repeat the assay. Multiply result by the dilution factor, *n*.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates, black flat-bottom uncoated 96-well plates, and plate reader.



Left: Uric Acid Optical Density Assay Standard Curve. ***Right***: Fluorimetric Assay Standardd Curve.

EXAMPLES: Assays were performed according to the standard protocol. A spot urine sample from an adult male: 6.1 ± 0.05 mM (Colorimetric Assay); Human serum: 280.2 ± 9.4 µM (Fluorimetric Assay); HepG2 cell culture media and SC cell lysate: < 4 µM (below detection limit).

LITERATURE

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.