Uric Acid Assay Kit

Uric Acid Assay Kit is a detection kit for the quantification of Uric Acid content in urine, serum and plasma.

Catalog number: ARG82025

Package: 96 wells

TABLE OF CONTENTS

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INTRODUCTION

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $C_5H_4N_4O_3$. It forms ions and salts known as urates and acid urates, such as ammonium acid urate. Uric acid is a product of the metabolic breakdown of purine nucleotides, and it is a normal component of urine. High blood concentrations of uric acid can lead to gout and are associated with other medical conditions, including diabetes and the formation of ammonium acid urate kidney stones.

In human blood plasma, the reference range of uric acid is typically 3.4–7.2 mg per 100 ml (200–430 µmol/l) for men, and 2.4–6.1 mg per 100 ml for women (140–360 µmol/l). Uric acid concentrations in blood plasma above and below the normal range are known as, respectively, hyperuricemia and hypouricemia. Likewise, uric acid concentrations in urine above and below normal are known as hyperuricosuria and hypouricosuria. Uric acid levels in saliva may be associated with blood uric acid levels.

High uric acid

Hyperuricemia (high levels of uric acid), which induces gout, has various potential origins:

- Diet may be a factor. High intake of dietary purine, high-fructose corn syrup, and table sugar can increase levels of uric acid.
- Serum uric acid can be elevated by reduced excretion via the kidneys.
- Fasting or rapid weight loss can temporarily elevate uric acid levels.
- Certain drugs, such as thiazide diuretics, can increase blood uric acid levels

by interfering with renal clearance.

- Tumor lysis syndrome, a metabolic complication of certain cancers or chemotherapy, due to nucleobase and potassium release into the plasma.

[Wikipedia Uric acid]

PRINCIPLE OF THE ASSAY

The kit is used to measure Uric Acid in urine, serum and plasma samples. Uric acid in samples is oxidized by uricase to form allantoin and hydrogen peroxide. The hydrogen peroxide reacts with a chromogen in the presence of peroxidase to form a quinone-imine dye. Quinone-imine dye is a red dye complex and it can be measured at 505 nm. The Uric Acid concentration in the sample is then determined by comparing the O.D. of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, Enzyme and Reaction Dye should be store at -20°C. Store all other components at 4°C at all time. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate	1 X 96-well plate	4°C
Standard (4 mmol/L)	1 ml (Ready to Use)	4°C
Diluent	20 ml (Ready to Use)	4°C
Enzyme	1 vial (Lyophilized)	-20°C, (Protect from light)
Reaction Dye	1 vial (Lyophilized)	-20°C, (Protect from light)
Plate sealer	3 strips	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 505 nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, Enzyme and Reaction Dye should be store at -20°C and protect from light. After reconstruction, Enzyme and Reaction Dye should be aliquoted and stored at-20°C to avoid repeated freeze-thaw cycles. . Store all other components at 4°C at all time.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Remove serum and assay immediately or aliquot and store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C or below. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Reaction Dye:** Reconstitute the Reaction Dye with 10 ml of Diluent. Allow the Reaction Dye to sit for few minutes with gentle agitation to make sure the Reaction Dye is dissolved completely before use. Aliquot & store the reconstituted Reaction Dye at -20°C. Store the reconstituted dye protect from light. Avoid repeated freeze-thaw cycles.
- **Enzyme:** Reconstitute the Enzyme with 10 ml of Diluent. Allow the Enzyme to sit for few minutes with gentle agitation to make sure the Enzyme is dissolved completely before use. Aliquot & store the reconstituted Enzyme at -20°C. Store the reconstituted Enzyme protect from light. Avoid repeated freeze-thaw cycles.
- **Sample**: Samples can be used directly. If the initial assay found samples contain Uric Acid Assay Kit higher than the standard (4 mmol/L), the samples can be diluted with distilled water and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use.

Standards and samples should be assayed in at least duplicates.

1. Add **100 µl** of reconstituted **Enzyme** per well into sample, Standard and blank well.
2. Add **100 µl** of reconstituted **Reaction Dye** per well into sample, Standard and blank well.
3. Add **10 µl** per well of **samples** and **standard** in duplicates into the appropriate wells in the plate.
4. Add **10 µl** per well of **distilled water** in duplicates into blank wells.
5. Gently tap the plate to ensure **thorough mixing**.
6. Incubate the plate at **37°C for 15 min in dark**.
7. Read the OD with a microplate reader at **505 nm** immediately.

Summary of Uric Acid Assay Procedure

Reagent	Sample	Standard	Blank
Enzyme	100 µl	100 µl	100 µl
Reaction Dye	100 µl	100 µl	100 µl
Sample	10 µl	-	-
Standard	-	10 µl	-
Distilled water	-	-	10 µl
Mix thoroughly, incubate the tubes at 37°C for 15 min in dark.			
Read the OD with a microplate reader at 505 nm immediately.			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standards and blank.

2. Calculation:

A. Definition:

$C_{Standard}$: the protein concentration, 4 mmol/L.

B. Formula:

Uric Acid (mmol/L) =

$C_{standard}$ X [(OD_{sample} – OD_{Blank}) / ($OD_{Standard}$ – OD_{Blank})]

= 4 X [(OD_{sample} – OD_{Blank}) / ($OD_{Standard}$ – OD_{Blank})]

3. Detection range:

The detection range is from 0.04 mmol/L to 4 mmol/L

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and serially diluted standards are necessary for this kit.