Uric Acid Assay Kit

Catalog No.: abx298824

Size: 96 tests

Detection Range: 1.30 mg/L – 80 mg/L

Sensitivity: 1.30 mg/L

Storage: Store all components in the dark at 2-8°C.

Application: For measurement of uric acid (UA) concentration in serum, plasma and urine samples.

Introduction

Uric acid is a purine metabolite, which gets degraded by uric acid enzymes into allantoin. Humans do not have the gene for uric acid oxidase, therefore, uric acid is the final product of purine metabolism. This means uric acid levels are higher in human blood than in other animals. Uric acid is a physiologically important plasma antioxidant that protects biological target from oxidation by hydroxy radicals, hypochloric acid and peroxynitrite. Uric acid will reduce phosphotungstic acid to tungsten blue, with an absorbance maximum at 690 nm..

Abbexa's Uric Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Uric Acid concentration. The product has an absorbance maxima at 690 nm. The intensity of the color is proportional to the concentration of Uric Acid, which can then be calculated.

Kit components

1. 96-well microplate
2. Uric Acid Standard (1 g/L): 1 ml
3. Protein Precipitation Solution: 30 ml
4. Alkaline Reagent: 6 ml
5. Phosphotungstic Acid: 6 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (690 nm)
2. Deionized water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Sonicating water bath
7. Centrifuge
8. Vortex mixer
9. Incubator (37°C)

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum/Plasma:** Samples can be tested directly. Assay immediately or aliquot and store at -80°C for up to 1 month
- **Urine:** Collect fresh urine into a sterile container, then centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Mouse Serum	1-2
Rat Serum	1
Human Serum	1
Pig Serum	1
Dog Serum	1-2
Human Urine	8-10

Note:

- Fresh samples or recently obtained samples are recommended to prevent denaturing that may lead to erroneous results.
- Ensure final supernatant is clear before testing.

2. Reagents

- **Standards:** Label 7 tubes with 80 mg/L, 60 mg/L, 50 mg/ml, 40 mg/L, 30 mg/L, 20 mg/L, and 10 mg/L. Add 80 µl, 60 µl, 50 µl, 40 µl, 30 µl, 20 µl, and 10 µl of Standard (1 g/L) to the 80 mg/L, 60 mg/L, 50 mg/ml, 40 mg/L, 30 mg/L, 20 mg/L, and 10 mg/L tubes respectively, followed by 920 µl, 940 µl, 950 µl, 960 µl, 970 µl, 980 µl, and 990 µl of deionized water, to prepare Standard Dilutions with concentrations 80 mg/L, 60 mg/L, 50 mg/ml, 40 mg/L, 30 mg/L, 20 mg/L, and 10 mg/L. These volumes are summarized in the following table:

Standard Dilution (mg/L)	80	60	50	40	30	20	10
1 g/L Standard (µl)	80	60	50	40	30	20	10
Deionized water (µl)	920	940	950	960	970	980	990

For the blank, or 0 mg/L standard, use pure deionized water. The volume of each standard will be 1000 µl.

Note:

- Equilibrate all reagents to room temperature before use.

B. Assay Procedure

1. Mark microcentrifuge tubes for each standard, sample, and blank. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 25 µl of each standard dilution into the standard tubes.
3. Add 25 µl of sample into the sample tubes.
4. Add 25 µl of deionized water into the blank tubes.
5. Add 250 µl of Protein Precipitation Solution to each tube and mix thoroughly using a vortex mixer.
6. Allow the tubes to stand for 5 minutes.
7. Centrifuge at 2000 × g for 5 minutes.
8. Assign and record microplate well positions for each standard, sample, and blank.
9. Add 160 µl of the supernatant to the corresponding wells of the microplate.
10. Add 50 µl of Alkaline Reagent and 50 µl of Phosphotungstic Acid to the standard and sample wells. Mix thoroughly and allow to stand at room temperature for 15 minutes.
11. Measure the OD value of each well at 690 nm with the microplate reader. The absorbance detection must be completed within 20 minutes.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula y=ax+b. Based on this curve, the concentration of Uric Acid in each sample well can be derived with the following formulae:

Serum, Plasma, and Urine samples:

$$\textbf{Uric Acid Concentration (mg/L)} = \frac{(\Delta \mathbf{A_{690}} - \boldsymbol{b})}{\boldsymbol{a}} \times \boldsymbol{f}$$

where:

ΔA_{690}	$OD_{Sample} - OD_{Blank}$
a	The gradient of the standard curve
b	The intercept of the standard curve
f	The dilution factor of samples

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.