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) content 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. Alkali Reagent: 6 ml
5. Phosphotungstic Acid: 6 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (690 nm)
2. Double distilled 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:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position at 25°C from 30 minutes. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 700-1000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and 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 15 min. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. If a sample type is being used that is not included in the manual, it is recommended to do a preliminary experiment to verify the validity.

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.
- The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)

B. Assay Procedure

1. Set standard and sample tubes and label accordingly.
2. Dilute 1 g/L of Uric Acid Standard with double distilled water to create a serial dilution. The recommended dilution gradient is: 0, 10, 20, 30, 40, 50, 60 and 80 mg/ml. Please see the below table as a reference.

Standard Concentrations (mg/L)	1 g/L Uric Acid Standard (µl)	Double Distilled Water (µl)
0	0	1000
10	10	990
20	20	980
30	30	970
40	40	960
50	50	950
60	60	940
80	80	920

3. Add 25 µl of standard into the Standard tubes.
4. Add 25 µl of sample into the Sample tubes.
5. Add 250 µl of Protein Precipitation Solution to each tube and mix with the vortex mixer.
6. Allow the tubes to stand for 5 minutes.
7. Centrifuge at 2000 × g for 5 minutes.
8. Set standard and sample wells on the 96 well microplate. It is recommended to measure standards in duplicate.. *Add the solution to the bottom of each well without touching the side walls. Pipette samples up and down to mix before adding to wells. Avoid foaming or bubbles.*
9. Add 160 µl of the supernatant to the corresponding wells of the microplate.
10. Add 50 µl of Alkali Reagent and 50 µl of Phosphotungstic Acid to the standard and sample wells. Mix and all 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

The standard curve can be plotted as the absolute OD_{450} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve (y = ax + b). Create the standard curve with graph software. The Uric acid concentration of the samples can be interpolated from the standard curve.

$$\text{Uric acid (mg/L)} = \frac{(\Delta A_{690} - b)}{a} \times f$$

where:

y	$OD_{Standard}$ - OD_{Blank}
x	The concentration of standard
a	The gradient of the standard curve
b	The intercept of the standard curve
ΔA_{690}	$OD_{Sample} - OD_{Blank}$
f	The dilution factor of samples
OD_{Sample}	Absorbance of sample well
$OD_{Standard}$	Absorbance of each standard
OD_{Blank}	Absorbance of the blank well