Uric Acid (UA) Assay Kit

Catalog No.: abx294015

Size: 100 tests

Detection Range: 0.58 mg/L – 100 mg/L

Sensitivity: 0.58 mg/L

Storage: Store all components at 4°C. Store the Phosphotungstic Acid Reagent in the dark.

Application: For detection and quantification of Uric Acid content in serum, plasma, and urine.

Introduction

Abbexa's Uric Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Uric Acid concentration. In protein free filtrate, Uric Acid reduces phosphotungstic acid to produce tungsten blue. The optical density of the blue color is proportional to the Uric Acid concentration, which can then be calculated.

Kit components

1. Alkaline Reagent: 60 ml
2. Phosphotungstic Acid Reagent: 60 ml
3. Protein Precipitator: 4 × 60 ml
4. Standard (1 g/L): 1 ml

Materials required but not provided

1. Spectrophotometer (690 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. 2.5 ml microcentrifuge tubes
7. 1 cm Optical path cuvettes
8. Centrifuge
9. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -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** and **Plasma:** Serum and plasma samples can be tested directly.
- **Urine:** Centrifuge at 10,000 × g 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
Human urine	8 – 10
Human serum	1
Dog serum	1
Rat serum	1
Mouse serum	1 – 2
Porcine serum	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- Urine supernatant should be clear and free of any particles after centrifugation.

2. Reagents

- **Standards:** Label 7 tubes with 10 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, and 100 mg/L. Prepare dilutions as summarized in the following table:

Standard Concentration (mg/L)	**10**	**40**	**60**	**80**	**100**
Standard (1 g/L) (µl)	10	40	60	80	100
Double-distilled water (µl)	990	960	940	920	900

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

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

The color stability of uric acid may be poor, so it is recommended to record colorimetric values within 20 minutes of the color development.

1. Mark microcentrifuge tubes for each standard concentration and sample. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 0.2 ml of sample to each sample tube.
3. Add 0.2 ml of each standard concentration to the corresponding standard tubes.
4. Add 2 ml of Protein Precipitator to all tubes. Vortex for at least 1 minute to mix fully.
5. Allow the tubes to stand at room temperature for 10 minutes.
6. Centrifuge at 1708 × g for 5 minutes. If the supernatant contains visible particles after centrifuging, transfer to a new tube and centrifuge again.
7. Aliquot 1.6 µl of supernatant to duplicate labelled tubes.
8. Add 0.5 ml of Alkaline Reagent to all tubes.
9. Add 0.5 ml of Phosphotungstic Acid Reagent to all tubes.
10. Mix thoroughly and allow tubes to stand at room temperature for 10 minutes.
11. Zero the spectrophotometer using double-distilled water. Measure the OD values for each tube at 690 nm using 1 cm optical path cuvettes.

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:

$$\text{Uric Acid Concentration (mg/L)} = \mathrm{F} \times \frac{(\mathrm{OD_{Sample}} - \mathrm{OD_{Blank}} - \mathrm{b})}{\mathrm{a}}$$

where:

$\mathrm{OD_{Sample}}$	OD value of sample
$\mathrm{OD_{Blank}}$	OD value of blank
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	The dilution factor of sample

Technical Support

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