Uric Acid (UA) Assay Kit

Catalog No.: abx295079

Size: 96 tests

Detection Range: 0.03 µmol/L – 15 µmol/L

Sensitivity: 0.03 µmol/L

Storage: Store all components at -20°C. Store the Probe Reagent in the dark.

Application: For the detection and quantification of Uric Acid (UA) concentration in serum, plasma, tissue homogenates, and urine.

For Reference Only

Principle of the Assay

Abbexa's Uric Acid (UA) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Uric Acid (UA) concentration. Uricase catalyzes the decomposition of uric acid into allantoin, CO_2, and H_2O_2. In the presence of peroxidase, the generated H_2O_2 oxidizes a non-fluorescent probe producing a fluorescent substance with an excitation wavelength of 535 nm and an emission wavelength of 587 nm. The fluorescence intensity is proportional to the uric acid concentration, which can then be calculated.

Kit components

1. Black 96-well microplate
2. Assay Buffer: 2 × 60 ml
3. Probe Reagent: 0.24 ml
4. Enzyme Reagent A: 0.24 ml
5. Enzyme Reagent B: 1.2 ml
6. Standard (20 µmol/L): 1.5 ml
7. Plate sealer: 2

Materials required but not provided

1. Fluorescence microplate reader (Excitation: 535 nm, Emission: 587 nm)
2. PBS (0.01 M, pH 7.4)
3. Pipette and pipette tips
4. Centrifuge and centrifuge tubes
5. Mechanical homogenizer
6. Vortex mixer
7. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Assay Working Solution**: Prepare enough Assay Working Solution for the wells to be tested. Mix Probe Reagent, Enzyme Reagent A, Enzyme Reagent B and Assay Buffer at a ratio of 1 : 1 : 5 : 18. For example, prepare 50 µl of Assay Working Solution by mixing 2 µl of Probe Reagent, 2 µl of Enzyme Reagent A, 10 µ of Enzyme Reagent B and 36 µl of Assay Buffer. This solution should be prepared immediately before use.
- **Standards:** Label 7 tubes with 2 µmol/L, 4 µmol/L, 6 µmol/L, 8 µmol/L, 10 µmol/L, 12 µmol/L, and 15 µmol/L. Prepare these dilutions according to the volumes in the following table:

Standard Dilution (µmol/L)	2	4	6	8	10	12	15
20 µmol/L Standard (µl)	20	40	60	80	100	120	150
Assay Buffer (µl)	180	160	140	120	100	80	50

For the blank, use pure Assay Buffer. The volume of each standard dilution will be 200 µl.

Note:

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

2. 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 and other liquid samples**: Samples can be tested directly. Prepared serum or plasma sample can be aliquoted and stored at -80°C for up to 1 month.
- **Tissue homogenates:** Carefully weigh at least 20 mg of tissue. Wash the tissue with cold PBS (0.01 M, pH 7.4). Per 20 mg of tissue, add 180 µl of Assay Buffer. Homogenize manually or use a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant for detection. Keep on ice and assay immediately.

Note: To calculate Uric Acid (UA) concentration in tissue homogenates using the formulae in section **C. Calculation of Results**, the total protein concentration of the supernatant must be determined separately (abx097193).

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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 Assay Buffer, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	10 – 20
Human urine	8 – 100
Human hydrothorax	50 – 60
Rat serum	10 – 20
Rat urine	10 – 20
Rabbit serum	5 – 10
Porcine serum	1
10% Rat liver tissue homogenate	10 – 20
10% Rat kidney tissue homogenate	30 – 40
10% Rat lung tissue homogenate	10 – 20

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 performance of this kit. It is recommended to use mechanical lysis methods only for tissue homogenates.

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B. Assay Procedure

Mark positions on the 96-well microplate for each standard, blank, and sample. *Each sample requires a corresponding control. It is strongly recommended to test all wells in duplicate*.

1. Add 50 µl of each standard dilution to the standard wells.
2. Add 50 µl of Assay Buffer to the blank wells.
3. Add 50 µl of sample to the sample wells.
4. Add 50 µl of Assay Working Solution to all wells.
5. Mix the well contents by tapping the plate, or shaking with a microplate shaker for approximately 5 seconds.
6. Cover with a plate sealer, then incubate at 37°C for 30 minutes.
7. Unseal the plate, then measure the fluorescence of each well with a fluorescence microplate reader with an excitation wavelength of 535 nm and an emission wavelength of 587 nm.

C. Calculation of Results

Plot the standard curve, using the mean fluorescence 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 (UA) in each sample well can be derived using the following formulae:

1. Serum, plasma, and other liquid samples:

$$\text{Uric Acid (µmol/L)} = \frac{(F_{Sample} - F_{Blank} - b)}{a} \times F$$

2. Tissue samples:

$$\text{Uric Acid (µmol/g protein)} = \frac{(F_{Sample} - F_{Blank} - b)}{a \times C_{Protein}} \times F$$

where:

F_{Sample}	Fluorescence value of the sample.
F_{Blank}	Fluorescence value of the blank
$C_{Protein}$	Concentration of protein in sample (µmol/L)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
F	Dilution factor of the sample

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Technical Support

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