MA-0151

Uric Acid Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Introduction:

Uric acid, a byproduct of purine metabolism, has both positive and negative aspects in metabolism. While elevated levels are associated with gout, a painful joint condition, uric acid serves essential functions, acting as an antioxidant, neutralizing harmful free radicals, and protecting cells from oxidative stress. It contributes to the regulation of blood pressure by modulating the production of nitric oxide, a vasodilator. Despite its physiological benefits, excessively high uric acid levels can lead to gout and other metabolic disorders such as insulin resistance, obesity, and cardiovascular disease. AkrivisBio's Uric Acid assay is a simple, sensitive means of measuring uric acid levels in a variety of biological samples with a sensitivity below 1 nmole.

Assay Principle:

1 – Uricase oxidizes uric acid forming 5-hydroxyisourate and hydrogen peroxide
2 – Peroxidase utilizes peroxide oxidizing ADHP to resorufin with a large increase in color (570 nm) and fluorescence (535/587 nm).

Assay Components:

Assay Buffer	25 ml	WM	MA-0151A
ADHP Solution	0.2 ml	Red	MA-0151B
Uricase/HRP	lyoph	Green	MA-0151C
Uric Acid Standard	1 ml	Yellow	MA-0151D

Storage and Handling Considerations:

Store assay at -20°C. Warm to room temperature before using.
Assay Buffer: Ready to use as supplied. Store at 4°C.
ADHP Solutions: Ready to use as supplied. Make sure DMSO melts before using. Store at -20°C.
Uricase/HRP: Reconstitute with 220 µl of Assay Buffer. Best to aliquot to convenient portions and store at -20°C.
Uric Acid Standard: Ready to use as supplied. Store at 4°C

Assay Protocol:

1. Standard Curve:

Absorbance-based assay: Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells of a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0 – 10 – 20 – 30 – 40 – 50 nmol of Uric Acid.

Fluorescence-based assay: Dilute the Uric Acid Standard 10X by adding 20 µl to 180 µl of Assay Buffer. Mix well. : Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells of a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0 – 1 – 2 – 3 – 4 - 5 nmol of Uric Acid.

2. Samples: Add up to 50 µl of liquid samples per well and bring all sample wells to 50 µl with Assay Buffer. Serum and urine normally contain ~0.2-0.5 mM uric acid, so 2-10 µl is sufficient. Readings must be within the range of the standard curve. If readings are outside of that range, the sample should be diluted appropriately and rerun.

3. Reaction Mix: Each sample and standard well requires 50 µl of Reaction Mix. Prepare sufficient Reaction Mix for the total number of wells being analyzed containing:

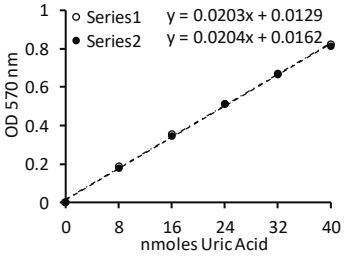
	Reaction Mix
Assay Buffer	46 µl
ADHP Solution	2 µl
Uricase/HRP	2 µl

Add 50 µl of Reaction Mix to each well .

4. **Measurement**: Monitor the reaction with the chosen detection system at 37°C for 30 minutes.

5. **Typical Results**:

	Standard Raw Values		Background Corrected Values	
nmole Standard	OD			
0	0.0712	0.0690	0	0
8	0.2582	0.2481	0.1870	0.1791
16	0.4251	0.4146	0.3539	0.3456
24	0.5828	0.5839	0.5116	0.5149
32	0.7394	0.7329	0.6682	0.6639
40	0.8913	0.8831	0.8201	0.8141



6. **Calculation:** Subtract the zero standard value from all other standard readings. Plot the Standard Curve. Determine the slope of the Standard Curve. This value (OD/nmol) defines the sensitivity of the measurement system.. Subtract the zero standard value from all test sample readings. Divide the background corrected test sample reading by the slope of the Standard Curve to get nmoles of uric acid in the sample wells. To convert back to uric acid concentration in the original samples:

A – Divide nmoles uric acid in the sample well by the volume of sample added to the well in µl = nmoles uric acid per µl of sample
B – Multiply by any dilution factor you might have diluted the sample by = corrected total nmoles per µl of sample

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