Uric Acid Assay (UA)

Cat. No. 8438
100 Tests in 96-well plate

Introduction

Uric acid is the end product of the metabolic breakdown of purine nucleotides. Uric acid is dissolved in the blood and passes through the kidneys into the urine. When more uric acid is produced than the kidneys can eliminate, the elevated level is known as hyperuricemia, which is associated with insulin resistance, cardiovascular disease, and gout. Increased serum uric acid can be a marker of renal disease. This colorimetric assay is based on uricase-catalyzed oxidation of uric acid, in which the formed hydrogen peroxide is catalyzed by peroxidase and reacts with 4-aminoantipyrine to form the product dye. The color intensity of the reaction product at 540nm is directly proportional to uric acid concentration in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8438a	1	Assay buffer	10 mL	4°C
8438b	1	Uric Acid standard	1 mL	-20°C
8438c	1	Substrate mix	1.6 mL	-20°C
8438d	1	Enzyme mix	0.2 mL	-20°C

Product Use

The Uric Acid Assay kit measures the uric acid level of different types of samples, such as serum, plasma, urine. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Data from Uric Acid Assay of uric acid solutions with concentrations ranging from 15 to 500 µM show a linear relationship between OD_{540nm} and uric acid concentration (Figure 1).

Shipping

Shipped on dry ice.

Procedure (96-well plate)

A. Preparation of uric acid standard

1. Obtain 7 test tubes, add 25 µL of assay buffer (8438a) into each tube and label them #1 through #7.
2. Add 25 µL 1 mM uric acid (8438b) into tube #1 and mix well to get the 500 µM uric acid standard.
3. Transfer 25 µL of the 500 µM uric acid standard from tube #1 to tube #2 and mix well to get the 250 µM uric acid standard.
4. Repeat step 4 for tubes #3-6 to serially dilute the uric acid standards. Do not add any uric acid to tube #7, which serves as blank.
5. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each uric acid standard by aliquoting 10 µL/well of each uric acid standard into duplicate wells of the 96-well test plate, according to the following plate format:

	#1	#2	#3	#4	#5	#6	#7
A	500µM	250µM	125µM	62.5µM	31.25µM	15.625µM	Blank
B	500µM	250µM	125µM	62.5µM	31.25µM	15.625µM	Blank

B. Preparation of test samples

Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well of reaction, prepare working reagent by mixing 72 µL assay buffer (8438a), 16 µL substrate mix (8438c) and 2 µL enzyme mix (8438d).
2. Add 90 µL of working reagent mix into each well of the 96-well plate containing uric acid standard, samples and blank. Incubate for 30 minutes at room temperature in dark.
3. Read the absorbance at 540 nm with an ELISA plate reader.

D. Calculations

1. Subtract the OD_{540nm} value of the blank from the OD_{540nm} values obtained with all other standard and samples to get ΔOD_{540nm} value.
2. Based on the calibrated ΔOD_{540nm} of the uric acid standard, make a standard curve by plotting ΔOD_{540nm} as a function of uric acid concentration (See Figure 1 for a typical standard curve). Determine the equation and R^2 value of the trend line.
3. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the uric acid concentration of test samples as follows:

$$[\text{Uric acid}] = \frac{\Delta OD_{540nm} - B}{A}$$

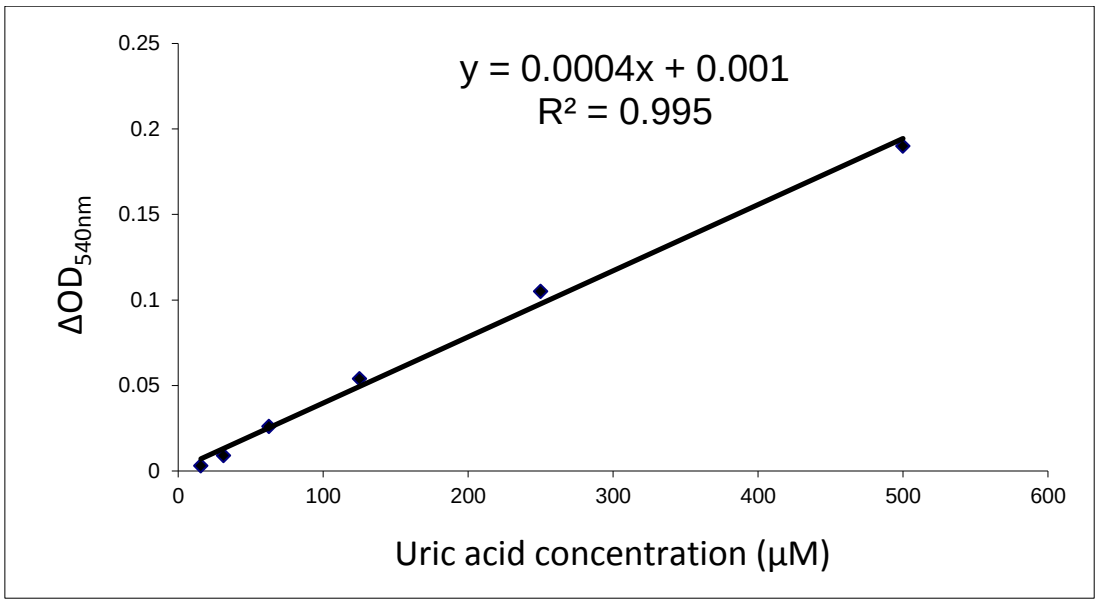

Figure1. A typical uric acid standard curve measured by ScienCell™ Uric Acid Assay kit