MANUAL

RayBio® Uric Acid Colorimetric Assay Kit

Catalog #: MA-UA

ISO 13485:2016

Introduction

Uric Acid serves as the end-product of human purine metabolism, resulting from the oxidation of xanthine and hypoxanthine by xanthine oxidase, and is subsequently excreted in urine. The kidney facilitates the clearance of uric acid through glomerular filtration. However, humans often lack the necessary enzyme called urate oxidase (uricase), and consequently abnormal uric acid levels may accumulate in the bloodstream. Elevated serum uric acid levels, known as hyperuricemia, are associated with insulin resistance, cardiovascular diseases, and gout. Furthermore, increased serum uric acid can serve as an indicator of renal disease.

RayBio® Uric Acid Colorimetric Assay Kit provides a simple, reproducible, and sensitive tool for measuring uric acid concentration in plasma, serum, cell lysates, urine and other biological liquid samples. This assay utilizes a coupled enzymatic reaction system. First, uricase catalyzes the conversion of uric acid to allantoin and hydrogen peroxide (H_2O_2). H_2O_2, in the presence of horseradish peroxidase, reacts with a colorimetric probe to form a pink-colored product. The optical density measured at 520nm is directly proportional to the uric acid concentration in the sample.

Storage

The entire kit may be stored at 2–8 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate (Item A)	A 96-well (12 strips x 8 wells) plate	RT*
Sample Buffer	10 ml	2–8 °C
Uric Acid Standard	1 vial (250 µl of 5 mg/dL)	2–8 °C
Enzyme Mix Solution	20 ml	2–8 °C

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 520 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. Incubator at 37°C

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Typically, Uric Acid levels in human plasma are less than 7 mg/dL (420 µM). Plasma samples can be diluted at least 1:2 with Sample Buffer.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, Uric Acid levels in human serum are in the range of 4-5 mg/dL (240-300 µM). Serum samples can be diluted at least 1:2 with Sample Buffer.

4. Urine samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. Urine samples can be diluted at least 1:10 with Sample Buffer.

NOTE:
Optimal experimental conditions for samples must be determined by the investigator. A set of serial dilutions is recommended for samples to achieve optimal assay results.

Standard Preparation

To prepare a dilution series of standard in the concentration range of 0 mg/dL – 5 mg/dL (see Table below),

1. Label 8 microtubes #1 through 8 which with the following concentrations: 5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, 0 mg/dL.
2. Pipette 200 µL Uric Acid Standard into tube #1, this is the 5 mg/dL standard.
3. Pipette 100µL Sample Buffer into labeled tubes #2 – tube #8.
4. To make the 2.5 mg/dL standard, pipette 100µL of tube #1 into the tube labeled #2. Mix thoroughly.
5. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 100 µl of the prior concentration until the 0.0781 mg/dL is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh, mixed thoroughly and used immediately.

Labeled Tubes	Uric Acid Standard (µL)	Sample Buffer (µL)	Standard Conc. (mg/dL)	Standard Conc. (µM)
1	200 µL	0µL	5	298
2	100 µL of Tube #1	100 µL	2.5	149
3	100 µL of Tube #2	100 µL	1.25	74.5
4	100 µL of Tube #3	100 µL	0.625	37.25
5	100 µL of Tube #4	100 µL	0.3125	18.625
6	100 µL of Tube #5	100 µL	0.1563	9.3125
7	100 µL of Tube #6	100 µL	0.0781	4.6563
8	0µL	100 µL	0	0

NOTE: 5 mg/dL Uric Acid is equal to 298 µM Uric Acid.

Assay Procedure

Each Uric Acid standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Pre-warm the Enzyme Mix Solution at 37°C for five minutes.
2. Add 40 µL of the diluted Uric Acid standards or samples to the 96-well microtiter plate.
3. Initiate the reaction by adding 200 µL of Enzyme Mix Solution to each well (pre-heat reagent at 37°C).
4. Cover with the plate cover. Carefully shake the plate for a few seconds to mix.
5. Incubate the plate for 10 minutes at 37°C.
6. Measure the absorbance at 520nm using a plate reader.

Calculation of Results

Subtract the blanks

Average the absorbance value of the blank wells (Standard 0 mg/dL) and subtract this from the absorbance values of all the other wells. These are the corrected absorbance.

Plotting the standard curves

Make a plot of corrected absorbance at 520nm as a function of Uric Acid concentration.

Determination of sample Uric Acid concentration

Uric Acid (mg/dL) = $\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$

OD_{Sample} = Optical density (OD) reading of the Sample

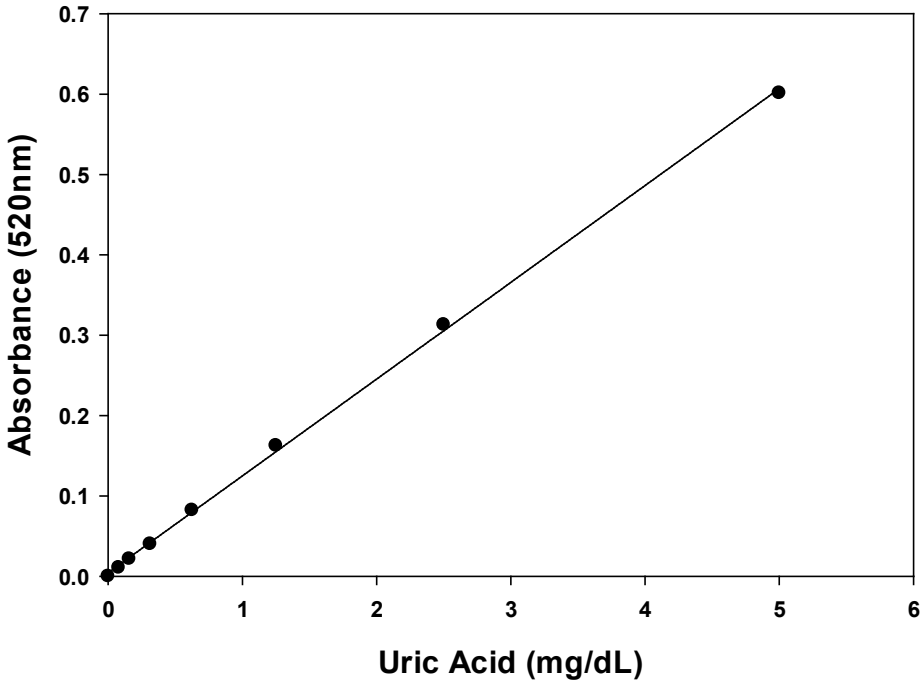
OD_{Blank} = Optical density (OD) reading of the Blank (Standard 0 mg/dL)

Slope is from the plot of Uric Acid concentration vs. Absorbance shown in Typical data below

DF = Sample Dilution factor (DF = 1 for undiluted samples)

Note: If the calculated Uric Acid concentration of the sample is higher than 5 mg/dL, dilute the sample in Sample Buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 0.99%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 2.6%.

This product is for research use only.