



Uric Acid

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8011

(Version 1.2A)

Detection and Quantification of Uric Acid (UA) Content in Urine,
Serum, Plasma, Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

In humans and primates, uric acid is the end product of purine metabolism. It is produced by the oxidation of xanthine and hypoxanthine by xanthine oxidase and excreted in urine. High serum levels of uric acid, hyperuricemia, are associated with insulin resistance, cardiovascular disease, and gout. The mechanisms leading to hyperuricemia are typically either increased uric acid production or decreased urine excretion. Increased serum uric acid may be a marker of renal disease.

Uric Acid Fluorometric Microplate Assay Kit provides a simple and sensitive method for monitoring uric acid concentration in various samples. The assay is initiated with the enzymatic catalysis of the uric acid by uricase, generating a compound that reacts with the probe, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Black Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 ml x 1	4 °C
Standard (0.5 mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dilute to 500 $\mu\text{mol/L}$ standard top solution by adding 500 μl 1 mM stock solution into 500 μl distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 500/250/125/62.5/31.2/15.6/7.8 $\mu\text{mol/L}$.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use. Aliquot & store at -20°C . Use within 1 month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 ml Probe Diluent to dissolve. Store at -20°C , protect from light and moisture. Use within 1 month.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For liquid samples

Detect directly or dilute with Assay Buffer.

VI. ASSAY PROCEDURE

Warm the solution to room temperature before use.

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	170 µl	170 µl	170 µl
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Probe	10 µl	10 µl	10 µl
Enzyme	10 µl	10 µl	10 µl
Mix, put it in the oven, 37 °C for 15 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**The concentrations can vary over a wide range depending on the different samples.

For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(\text{RFU}_{\text{Sample}} - \text{RFU}_{\text{Blank}}) - \text{Intercept}}{\text{Slope}} \times n (\mu\text{mol/L})$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard RFU and concentration

According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{RFU}_{\text{Sample}} - \text{RFU}_{\text{Blank}})}{(\text{RFU}_{\text{Standard}} - \text{RFU}_{\text{Blank}}) \times V_{\text{Sample}}} (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, $\mu\text{mol/L}$

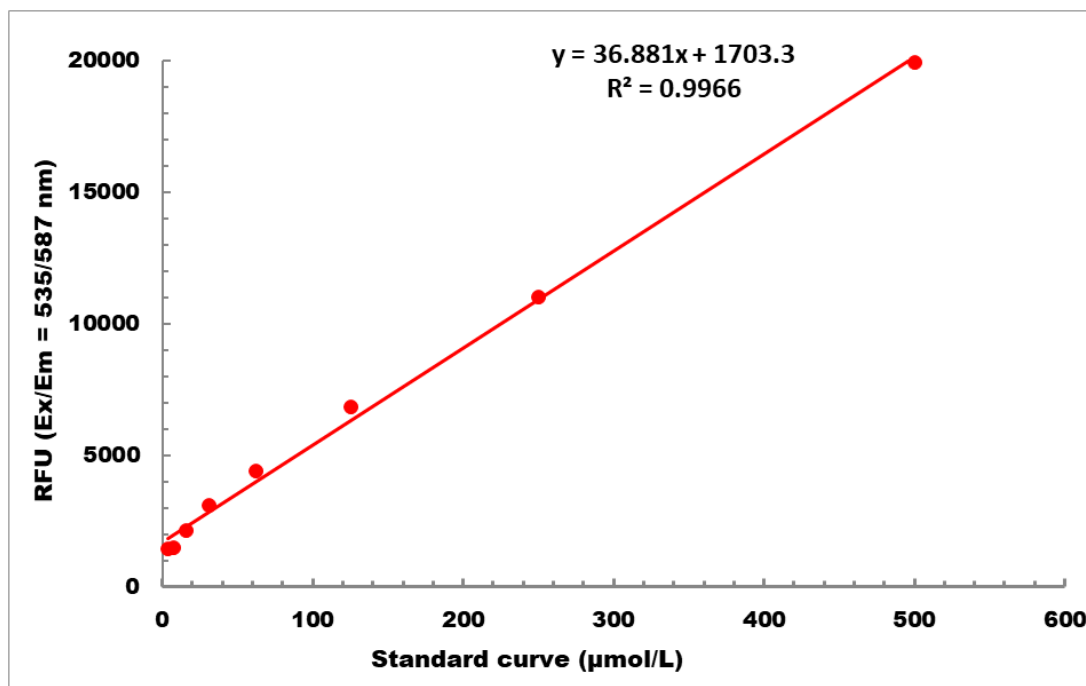
V_{Standard} : the volume of standard in assay procedure, μl

V_{Sample} : the volume of sample in assay procedure, μl

V_{Assay} : the volume of Assay Buffer, μl

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 5µmol/L -500µmol/L