

Colorimetric or fluorometric detection of uric acid in biological samples such as serum and urine

Instruction Manual

Catalog Number	PK-CA577-K608																	
Description	<p>Uric acid in serum is the end product of purine metabolism, and is cleared through the kidney by glomerular filtration. However, humans often lack the necessary enzyme called urate oxidase (Uricase), and therefore abnormal uric acid may be accumulated in blood. Recent evidences show the close association between serum urate level and cardiovascular morbidity and mortality, especially among persons at high cardiovascular risk, including those with hypertension, diabetes and congestive heart failure.</p> <p>PromoKine's Uric Acid Assay Kit provides a convenient means for detecting uric acid in biological samples such as serum and urine. Pretreatment of samples are not required. Uric acid level can be measured using fluorometric (at Ex/Em = 535/587 nm) or colorimetric (at $\lambda= 570$ nm) methods.</p>																	
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Kit Components	<table border="1"> <thead> <tr> <th>Components</th> <th>Quantity</th> <th>Color Code</th> </tr> </thead> <tbody> <tr> <td>Uric Acid Assay Buffer</td> <td>25 ml</td> <td>WM</td> </tr> <tr> <td>Uric Acid Probe (in DMSO, anhydrous)</td> <td>200 μl</td> <td>Red</td> </tr> <tr> <td>Uric Acid Standard (2 nmol/μl)</td> <td>1 ml</td> <td>Yellow</td> </tr> <tr> <td>Uric Acid Enzyme Mix (lyophilized)</td> <td>1 vial</td> <td>Green</td> </tr> </tbody> </table>	Components	Quantity	Color Code	Uric Acid Assay Buffer	25 ml	WM	Uric Acid Probe (in DMSO, anhydrous)	200 μ l	Red	Uric Acid Standard (2 nmol/ μ l)	1 ml	Yellow	Uric Acid Enzyme Mix (lyophilized)	1 vial	Green		
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Storage and Reagents Preparation	<p>Store the kit at -20°C, protected from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.</p> <ul style="list-style-type: none"> • Probe: Briefly warm at 37°C for 1-2 minutes to dissolve. Mix well, store at -20°C. Protect from light and moisture. Use within two months. • Uric Acid Enzyme Mix: Dissolve in 220 μl Uric Acid Assay Buffer. Pipet up and down to dissolve it completely. Store at -20°C. Use within two months. 																	
Assay Protocol	<p>1. Standard Curve Preparations:</p> <p><u>For colorimetric assay,</u> add 0, 4, 8, 12, 16, 20 μl into each well individually. Adjust volume to 50 μl/well with Uric Acid Assay Buffer to generate 0, 8, 16, 24, 32, 40 nmol/well of Uric Acid Standard.</p> <p><u>For fluorometric assay,</u> dilute the Uric Acid to 0.2 nmol/μl by adding 20 μl into 180 μl of Uric Acid Assay Buffer. Mix well. Add 0, 4, 8, 12, 16, 20 μl into each well individually. Adjust volume to 50 μl/well with Uric Acid Assay Buffer to generate 0, 0.8, 1.6, 2.4, 3.2, 4.0 nmol/well of the Uric Acid Standard.</p> <p>2. Sample Preparations: Prepare test samples in 50 μl/well with Uric Acid Assay Buffer in a 96-well plate. If using serum sample, serum (2-20 μl/assay, normal serum contains \sim0.3 nmol/μl uric acid) can be directly diluted in the Uric Acid Assay Buffer. Urine sample can be assayed directly without pre-treatment. We suggest using several dilutions to ensure that the readings are within the standard curve range.</p> <p>3. Reaction Mix Preparation: Mix enough reagents for the number of assays performed: For each well, prepare a total 50 μl Reaction Mix containing:</p> <ul style="list-style-type: none"> 46 μl Uric Acid Assay Buffer 2 μl Uric Acid Probe 2 μl Uric Acid Enzyme Mix <p>Mix well. Add 50 μl of the Reaction Mix to each well that contains the uric acid standard and test samples. Incubate the reaction for 30 min at 37°C, protect from light.</p> <p>4. Measurement: Measure OD 570nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader.</p> <p>5. Calculation: Correct background by subtracting the reading of no uric acid control from all standard and sample readings (The background reading can be significant and must be subtracted from sample readings). Then apply the sample reading to the standard curve.</p> <p>Uric Acid Concentration C = A/V x 1000 (nmol/ml)</p> <p>Where: A is the uric acid amount from the sample well in nmol.</p>																	

V is the sample volume added into the sample well in microliter(s).
 Uric acid molecular weight is 168.

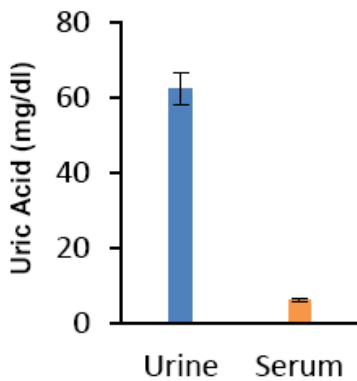
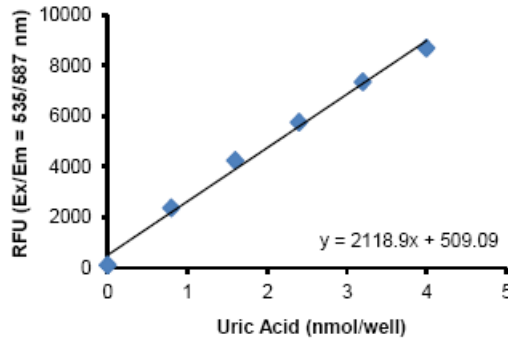
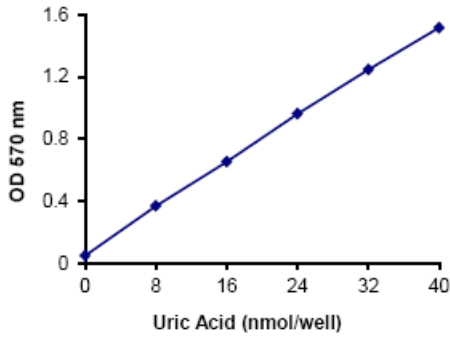


Figure. Uric acid Standard Curve. (a) Colorimetric. (b) Fluorometric. (c) Quantitation of Uric Acid concentration in human urine (25 µl, 50 times diluted) and serum (25 µl, undiluted).

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