

Uric Acid Colorimetric/Fluorometric Assay Kit

(Catalog #K608-100; 100 assays; Store kit at -20°C)

I. Introduction:

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. BioVision'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.

II. Kit Contents:

Components	100 Assays	Cap Color	Part Number
Uric Acid Assay Buffer	25 ml	WM	K608-100-1
Uric Acid Probe (in DMSO, anhydrous)	0.2 ml	Red	K608-100-2A
Uric Acid Enzyme Mix	1 Vial	Green	K608-100-4
Uric Acid Standard (2 nmol/ μ l)	1 ml	Yellow	K608-100-5

III. Reagent Preparation and Storage Conditions:

Probe: Briefly warm at 37°C for 1-2 min to dissolve. Mix well, store at -20°C. Protect from light and moisture. Use within two months.

Uric Acid Enzyme Mix: Dissolve in 220 μ l Uric Acid Assay Buffer. Pipet up and down to dissolve it completely. Store at -20°C. Use within two months.

IV. Uric Acid Assay Protocol:

- Standard Curve Preparations:** For colorimetric assay, add 0, 4, 8, 12, 16, 20 μ l into each well individually. Adjust volume to 50 μ l/well with Uric Acid Assay Buffer to generate 0, 8, 16, 24, 32, 40 nmol/well of Uric Acid Standard.

For fluorometric assay, dilute the Uric Acid to 0.2 nmol/ μ l by adding 20 μ l into 180 μ l of Uric Acid Assay Buffer. Mix well. Add 0, 4, 8, 12, 16, 20 μ l into each well individually. Adjust volume to 50 μ l/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.

- Sample Preparations:** Prepare test samples in 50 μ l/well with Uric Acid Assay Buffer in a 96-well plate. If using serum sample, serum (2-20 μ l/assay, normal serum contains ~0.3 nmol/ μ l 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.
- Reaction Mix Preparation:** Mix enough reagents for the number of assays performed: For each well, prepare a total 50 μ l Reaction Mix containing:

- 46 μ l Uric Acid Assay Buffer
- 2 μ l Uric Acid Probe
- 2 μ l Uric Acid Enzyme Mix

- Mix well. Add 50 μ l 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.
- Measure OD 570nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader.
- 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.

$$\text{Uric Acid Concentration } C = A/V \times 1000 \text{ (nmol/ml)}$$

Where: A is the uric acid amount from the sample well in nmol.

V is the sample volume added into the sample well in microliter(s).

Uric acid molecular weight is 168.

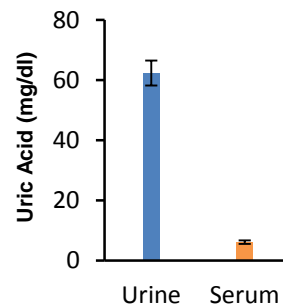
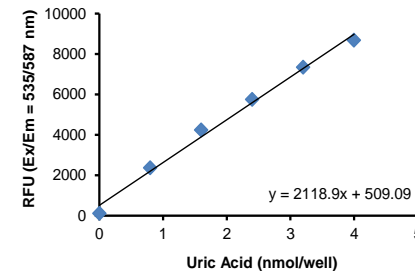
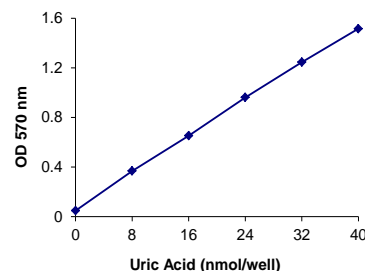


Figure. Uric acid Standard Curve. (a) Colorimetric. (b) Fluorometric. (c) Quantitation of Uric Acid concentration in human urine (25 μ l, 50 times diluted) and serum (25 μ l, undiluted).

RELATED PRODUCTS:

- Glucose, sucrose assay kit
- Lactate assay kit
- NAD+/NADH assay kit
- NADP+/NADPH assay kit
- Antibodies

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.