Uric Acid Colorimetric/Fluorometric Assay Kit

(Catalog #BN00832; 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. Assay Genie'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 λ = 570 nm) methods.

II. Kit Contents:

Components	100 Assays	Cap Color	Part Number
Uric Acid Assay Buffer	25 ml	WM	BN00832-1
Uric Acid Probe (in DMSO, anhydrous)	0.2 ml 1 Vial	Red Green	BN00832-2A BN00832-4
Uric Acid Enzyme Mix	1 ml	Yellow	BN00832-5
Uric Acid Standard (2 nmol/µl)			

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 µl 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:

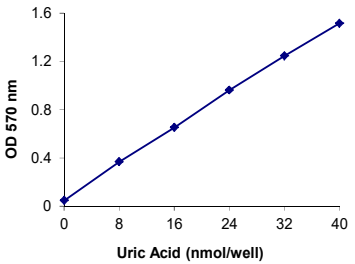
1. **Standard Curve Preparations:** For colorimetric assay, add 0, 4, 8, 12, 16, 20 µl into each well individually. Adjust volume to 50 µl/well with Uric Acid Assay Buffer to generate 0, 8, 16, 24, 32, 40 nmol/well of Uric Acid Standard.

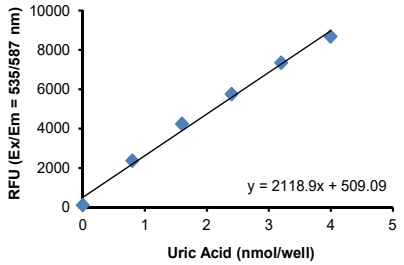
   For fluorometric assay, dilute the Uric Acid to 0.2 nmol/µl by adding 20 µl into 180 µl of Uric Acid Assay Buffer. Mix well. Add 0, 4, 8, 12, 16, 20 µl into each well individually. Adjust volume to 50 µl/well with Uric Acid Assay Buffer to generate 0, 0.8, 1.6, 2.4, 3.2, 4.0 nmol/well of the Uric Acid Standard.
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 ~0.3 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.
3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays performed: For each well, prepare a total 50 µl Reaction Mix containing:

   46 µl Uric Acid Assay Buffer
   2 µl Uric Acid Probe
   2 µl Uric Acid Enzyme Mix
4. 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.
5. Measure OD 570nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader.
6. 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.

   Uric Acid Concentration C = A/V x 1000 (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.





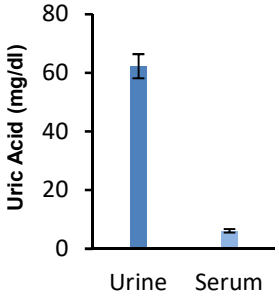


Figure. Uric acid Standard Curve. (a) Colorimetric. (b) Fluorometric. (c) Quantitation of Uric Acid concentration in human urine (25 µl, 50 times diluted) and serum (25 µl, undiluted).

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

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		