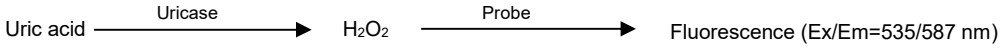
Uricase Activity Assay Kit (Fluorometric) (#BN00953)

(Catalog #BN00953; 100 assays; Store at -20°C)

I. Introduction:

Uricase (factor-independent urate hydroxylase, urate oxidase (UO); EC 1.7.3.3) is an enzyme involved in the uric acid metabolism. Uric acid is the end product of purine metabolism, and high levels of uric acid in blood causes gout. Uricase is present in a wide range of mammals but absent from human beings. It has been widely used for the measurement of uric acid concentrations in biological samples and recombinant uricase (rasburicase) has been used as a drug for the prevention of tumor lysis syndrome. Assay Genie's Uricase Activity Assay Kit provides a quick and easy method for the measurement of uricase activity in a wide variety of samples. In this assay, uricase oxidizes uric acid forming a product that reacts with the probe, thus generating a fluorescence signal (Ex/Em= 535/587nm). The generated fluorescence signal is directly proportional to the amount of active uricase present in the sample. This kit provides a sensitive and high throughput adaptable assay and can measure uricase activity in biological samples as low as 10 µU.



II. Applications:

- Measurement of uricase activities in various biological samples/preparations
- Analysis of uric acid degradation pathway and Urea Cycle

III. Sample Type:

- Mammalian tissues
- Plant tissue
- Purified enzyme

IV. Kit Contents:

Components	BN00953	Cap Code	Part Number
Uricase Assay Buffer	50 ml	NM	BN00953-1
Uricase Substrate	10 ml	WM	BN00953-2
Uricase Probe (in DMSO)	200 µl	Red	BN00953-3
Uricase Enzyme Mix	1 vial	Green	BN00953-4
H_2O_2 Standard (0.88 M)	100 µl	Yellow	BN00953-5
Uricase Positive Control	1 vial	Blue	BN00953-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- For Plants tissue: Liquid nitrogen, Dounce Tissue Homogenizer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay. Use within two months of opening.

- **Uricase Assay Buffer:** Warm to room temperature before use. Store at 4ºC or -20ºC.
- **Uricase Substrate:** Warm to room temperature before use. Aliquot and store at -20ºC. Vortex before use.
- **Uricase Probe (in DMSO):** Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Avoid from light.
- **Uricase Enzyme Mix:** Reconstitute with 220 µl Uricase Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.
- **H_2O_2 Standard (0.88 M):** Ready to use as supplied. Keep on ice while in use. Store at -20°C.
- **Uricase Positive Control:** Reconstitute with 1 ml Uricase Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

VII. Uricase Detection Assay Protocol:

1. **Sample Preparation: For plant tissue samples:** Grind tissue samples in liquid nitrogen. Rapidly homogenize tissue (~25 mg) with 100 µl ice cold Uricase Assay Buffer, and keep on ice for 10 min. Centrifuge at 16,000 x g for 10 min at 4°C to remove cell debris and collect the supernatant. Small molecules can interfere with the uricase activity, ultrafilter the samples with 10 kDa Spin Column under 16,000 x g for 15 min at 4°C. Wash the retentate with 100 µl Uricase Assay Buffer three times under 16,000 x g for 15 min at 4°C. Collect the retentate and bring the volume of the retentate back to the original sample volume. Add 2-10 µl of samples to designated wells of a 96-well clear plate. **For mammalian tissue samples:** Rapidly homogenize tissue (~20 mg) with 100 µl ice cold Uricase Assay Buffer, and keep on ice for 10 min. Centrifuge at 16,000 x g for 10 min at 4°C to remove cell debris and collect the supernatant. Add 80 µl saturated ammonium sulfate solution per 100 µl lysate. Keep on ice for 30 min. Centrifuge at 16,000 x g for 10 min at 4°C and discard the supernatant. Resuspend the precipitated protein with Uricase Assay buffer in the original volume of the lysate used. Dilute the samples 5-fold and add 2-10 µl of diluted samples to designated wells of a 96-well clear plate. For all samples, prepare Sample Background Control wells by adding the same amount of samples in parallel wells. **For positive control:** Dilute reconstituted Uricase Positive control by adding 10 µl of the positive control into 990 µl of Uricase Assay Buffer to make a 20X positive control stock. Further dilute the positive control by adding 10 µl of the 20X stock into 190 µl of the assay buffer. Add 10-20 µl of the diluted Uricase Positive Control. *Do not store*

the diluted positive control. Adjust the volume of Positive Control, Sample Background Control and Sample wells to 20 µl/well with Uricase Assay Buffer.

Notes: For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

2. **Standard Curve Preparation:**

   Add 10 µl of the 0.88 M H_2O_2 standard to 870 µl of dH_2O to generate 10 mM H_2O_2 standard. Further dilute the 10 mM standard by adding 10 µl of the 10 mM H_2O_2 standard into 990 µl dH_2O to make a 0.1 mM H_2O_2 standard. Mix well. Add 0, 2, 4, 6, 8, 10 µl of the 0.1 mM standards into a 96-well plate to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well. Bring the volume to 20 µl with the Uricase Assay buffer. *Do not store the diluted standards.*

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Prepare a 5-fold dilution of Uricase Probe (i.e. Mix 20 µl of Uricase Probe with 80 µl Uricase Assay Buffer). For each well, prepare 80 µl Mix containing:

	Reaction Mix	Background Mix
Uricase Assay Buffer	----	76 µl
Uricase Substrate	76 µl	----
Diluted Uricase Probe	2 µl	2 µl
Uricase Enzyme Mix	2 µl	2 µl

   Mix and add 80 µl of the Reaction Mix to each well containing the Standard Curve, test samples and positive control. Add 80 µl of Background mix to the sample background control.

   Note: Do not store Diluted Uricase Probe. Prepare fresh dilutions as needed.

4. **Measurement:** Measure fluorescence (Ex/Em=535/587nm) immediately in a microplate reader in kinetic mode for 30-45 min at 30 °C.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the H_2O_2 Standard Curve. If sample background control reading is significant, subtract the sample background control reading from its paired sample reading. Select the linear portion of the kinetic curve for urease activity calculation. Apply Sample ΔRFU ($RFU_2 - RFU_1$) to H_2O_2 Standard Curve to get B nmol of product generated during the reaction time ($\Delta t = t_2 - t_1$).

Sample Uricase Activity = B/(Δt X V) x D = nmol/min/ml = mU/ml

Where: **B** = H_2O_2 amount from Standard Curve (nmol)
Δt = reaction time (min)
V = sample volume added into the reaction well (ml)
D = Dilution Factor

The specific activity in biological samples can be expressed as U/mg of protein.

Unit Definition: One unit of uricase is the amount of enzyme that generates 1.0 µmol of H_2O_2 per min at pH 7.5 at 30°C.

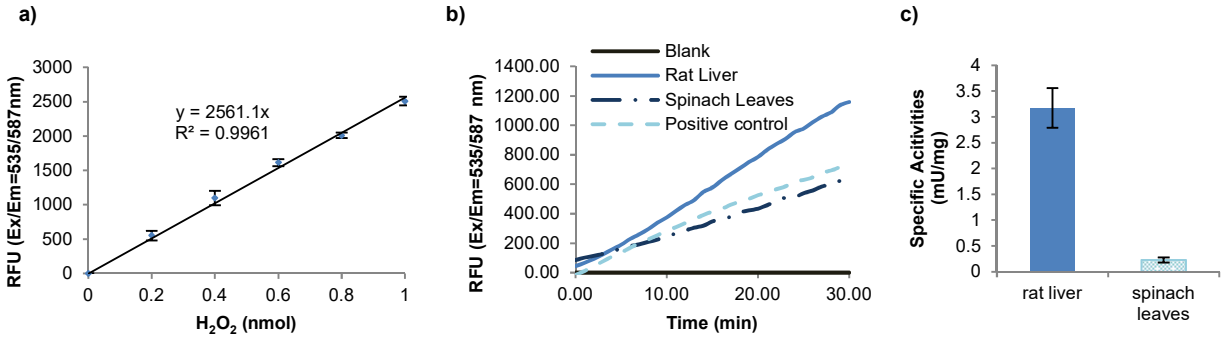


Figure: (a) Uricase Assay Standard curve; (b) Uricase activities in Rat liver (5.57 µg protein), Spinach leaves (31.5 µg protein) & Uricase positive control; (c) Specific Uricase activities in Rat liver and Spinach leaves. Assays were performed following the kit protocol.

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