Xanthine/Hypoxanthine Colorimetric/Fluorometric Assay Kit (#BN00909)

(Catalog # BN00909; 100 assays; Store at -20°C)

I. Introduction:

Xanthine, a catabolic product of purine metabolism, is present in body fluids, muscle tissue and certain plants. Structurally like caffeine, Xanthine has a stimulant effect and is used clinically to treat the congestive diseases such as asthma and chronic obstructive pulmonary disease. Xanthine is metabolized into uric acid and superoxide by Xanthine oxidase. Xanthine oxidase deficiency causes the rare genetic disorder-Xanthinuria, and leads to Xanthine accumulation in urine and blood, which ultimately progresses to renal failure. Recent studies show that Xanthine levels are elevated following ischemic injury, thus Xanthine can serve as a useful marker for tissue hypoxia. Early detection of Xanthine alteration in biological fluids is crucial for metabolic studies and for diagnostic and therapeutic monitoring. In Assay Genie's Xanthine/Hypoxanthine Assay kit, Xanthine/Hypoxanthine is specifically oxidized by the Xanthine Enzyme Mix to form an intermediate, which reacts with Developer & Probe to form a product that can be measured colorimetrically (λ = 570 nm) or fluorometrically (Ex/Em = 535/587 nm). Xanthine/Hypoxanthine Assay kit is rapid, simple and sensitive. This high-throughput suitable assay kit can detect Xanthine levels as low as 0.4 µM in various biological samples.

$$\text{Xanthine/Hypoxanthine} \xrightarrow{\text{Xanthine Enzyme Mix}} \text{Intermediate}$$

$$\text{Intermediate} \xrightarrow{\text{Probe + Developer}} \text{Color } (\lambda = 570 \text{ nm}) \text{ or Fluorescence (Ex/Em = 535/587 nm)}$$

II. Application:

- Measurement of Xanthine/Hypoxanthine in various tissues/cells and body fluids.
- Analysis of purine metabolism and cell signaling.

III. Sample Type:

- Body Fluids: serum, plasma, urine etc.
- Animal tissues: liver, muscle, heart etc.
- Cell culture: adherent or suspension cells.
- Cell and tissue culture supernatant.

IV. Kit Contents:

Components	BN00909	Cap Code	Part Number
Xanthine Assay Buffer	25 ml	WM	BN00909-1
GenieRed Probe (in DMSO)	0.2 ml	Red	BN00909-2A
Xanthine Enzyme Mix (Lyophilized)	1 vial	Blue	BN00909-3
Developer (Lyophilized)	1 vial	Green	BN00909-4
Xanthine Standard (Lyophilized)	1 vial	Yellow	BN00909-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate (colorimetric) or white plate (fluorometric) with flat bottom.
- Multi-well spectrophotometer (ELISA reader).

VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening.

VII. Reagent Preparation and Storage Conditions:

Xanthine Enzyme Mix: Reconstitute with 220 µl Xanthine Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze thaw. Keep on ice while in use. Use within two months.

Developer: Reconstitute with 220 µl Xanthine Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Use within two months.

Xanthine Standard: Reconstitute with 500 µl dH_2O to generate 2 mM (2 nmol/µl) Xanthine Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

VIII. Xanthine Assay Protocol:

1. **Sample Preparation:** Liquid samples can be measured directly. Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold Xanthine Assay Buffer for 10 minutes on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Add1-50 µl sample per well. Adjust volume to 50 µl with Xanthine Assay Buffer.

   Notes:

   A. Some enzymes in samples may interfere with the assay. Enzymes can be removed by 10 K quick spin columns or by perchloric acid/KOH treatment.

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

   C. For samples having high background, prepare parallel sample well(s) as background control.

2. **Standard Curve Preparation:** For colorimetric assay, add 0, 2, 4, 6, 8 & 10 µl of 2 mM Xanthine Standard into series of wells in 96 well plate to generate 0, 4, 8, 12, 16 & 20 nmol/well Xanthine Standard. Adjust volume to 50 µl per well with Xanthine Assay Buffer.

   For fluorometric assay, dilute Xanthine Standard to 0.02 mM (20 pmol/µl) by adding 10 µl of 2 mM Xanthine Standard to 990 µl dH_2O &

mix. Add 0, 2, 4, 6, 8 & 10 µl of 0.02 mM Xanthine Standard into series of wells in 96 well plate to generate 0, 40, 80, 120, 160 & 200 pmol/well Xanthine Standard. Adjust volume to 50 µl per well with Xanthine Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays (samples & Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	Background Control Mix*
Xanthine Assay Buffer	44 µl	46 µl
Xanthine Enzyme Mix	2 µl	------
Developer	2 µl	2 µl
GenieRed Probe	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well containing the Standard & test samples. Mix well.

***Notes:**

A. For samples having high background, add 50 µl of the Background Control Mix to sample background control well(s). Mix well.

B. The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.2 µl of the probe per reaction & adjust assay buffer to 45.8 µl.

4. **Measurement:** Incubate for 30 minutes at room temperature, protected from light. Measure fluorescence at Ex/Em = 535/587 nm or color at λ = 570 nm.

5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Xanthine Standard Curve. For samples having high background, correct sample background by subtracting the value derived from the background control from sample readings. Apply the corrected sample reading to the Xanthine Standard Curve to get B pmol or nmol of Xanthine/Hypoxanthine in the sample(s).

Xanthine/Hypoxanthine concentration in the sample **= B/V x Dilution Factor = nmol/ml or pmol/ml = µM or nM**

Where: **B** is the amount of Xanthine/Hypoxanthine in the sample (pmol or nmol)
V is the volume added to the reaction well (ml)
Xanthine molecular weight: 152.11 g/mol. Hypoxanthine molecular weight: 136.11g/mol
Xanthine/Hypoxanthine in samples can also be expressed in nmol/mg of sample or other desired method.

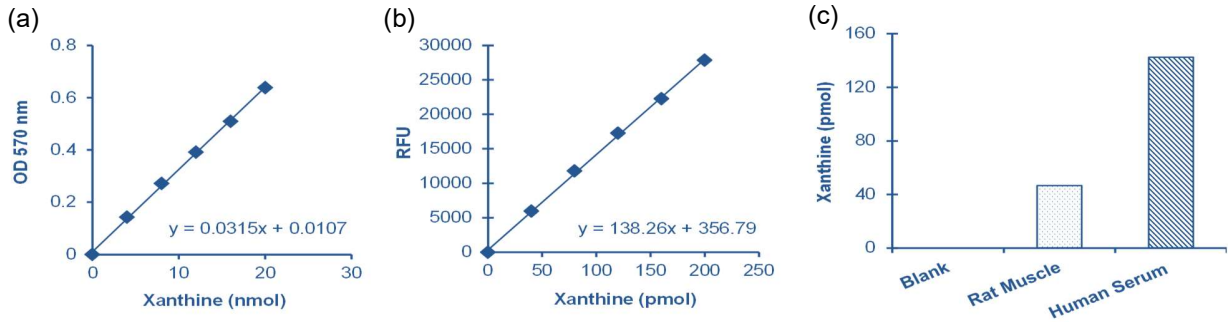

Figure 1. Xanthine Standard Curve (a) & (b). Measurement of Xanthine in rat muscle (1 µg) and human serum (2 µl) samples (c). Assays were performed following Kit protocol.

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