RayBio® Xanthine/Hypoxanthine Assay Kit

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RayBio® Xanthine/Hypoxanthine Assay Kit Protocol

(Cat#: 68-Xanthine-S100)

RayBiotech, Inc.

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Tel:(Toll Free)1-888-494-8555 or 770-729-2992; Fax:770-206-2393;
Web: www.raybiotech.com Email: info@raybiotech.com

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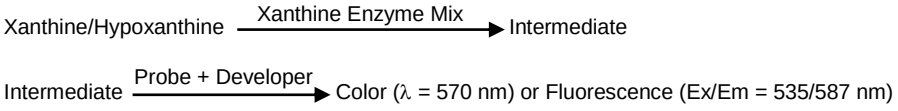
I. INTRODUCTION

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 RayBiotech'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). Our 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.



II. REAGENTS

Components	XAN-S100	Cap Code	Part Number
Xanthine Assay Buffer	25 ml	WM	Item A
OxiRed™ Probe (in DMSO)	0.2 ml	Red	Item B
Xanthine Enzyme Mix (Lyophilized)	1 vial	Blue	Item C
Developer (Lyophilized)	1 vial	Green	Item D
Xanthine Standard (Lyophilized)	1 vial	Yellow	Item E

III. SAMPLE TYPES

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

IV. REAGENT STORAGE & PREPARATION

- Store kit at –20°C, protected from light prior to use.
- Breifly centrifuge vials prior to use.
- Xanthine Assay Buffer: Warm to room temperature before use.
- 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 thaws. 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 dH2O to generate 2 mM (2 nmol/µl) Xanthine Standard solution. Keep on ice while in use. Store at –20°C. Use within two months.

V. ASSAY PROCEDURE:

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. Add 1-50 µl sample per well. Adjust volume to 50 µl with Xanthine Assay Buffer.

NOTES:

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

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

Some enzymes in samples may interfere with the assay. Enzymes can be removed by perchloric acid/KOH treatment if needed.

2. **Standard Curve Preparation:**

   a. For colorimetric assay, add 0, 2, 4, 6, 8 & 10 µl of 2 mM Xanthine Standard into series of wells in a 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.

   b. 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 dH2O & mix. Add 0, 2, 4, 6, 8 & 10 µl of 0.02 mM Xanthine Standard into series of wells in a 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 to be performed (samples & Standards). 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
OxiRedTM Probe ***	2 µl	2 µl

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

**** - 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.*

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

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

NOTE: Xanthine/Hypoxanthine in samples can also be expressed in nmol/mg of sample or other desired method.

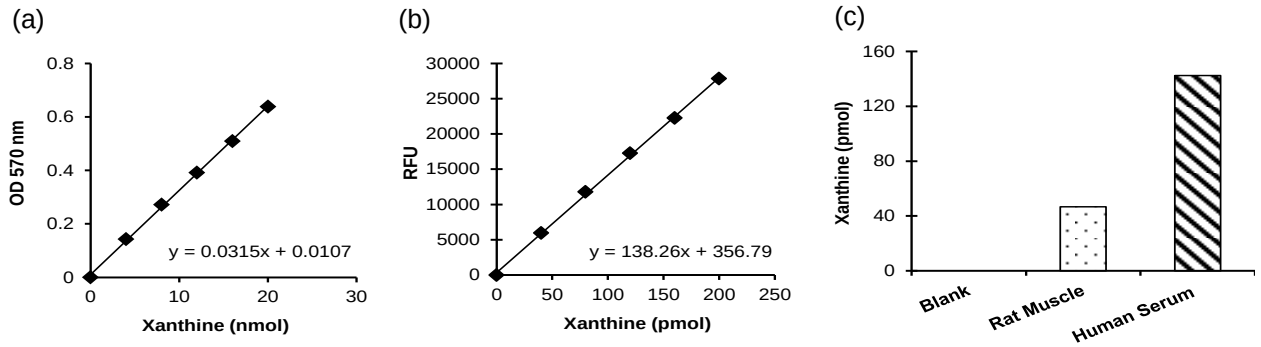


Figure 1. Xanthine Standard Curve (a) & (b). Measurement of Xanthine in rat muscle (1 µg) and human serum (2 µl) samples (c). Assays were performed following Kit protocol.

This product is for research use only.