Xanthine/Hypoxanthine Microplate Assay Kit User Manual

Catalog # CAK1228

(Version 1.2A)

Detection and Quantification of Xanthine/Hypoxanthine Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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.

Xanthine/Hypoxanthine Microplate Assay Kit is a sensitive assay for determining Xanthine/Hypoxanthine content in various samples. Xanthine/Hypoxanthine is specifically oxidized by the xanthine enzyme to form an intermediate, which reacts with Dye Reagent to form a product that can be measured at 505 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Enzyme	50 µl x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Enzyme: add 1 ml Reaction Buffer to dissolve before use.

Dye Reagent: add 10 ml Reaction Buffer to dissolve before use.

Standard: add 1 ml Assay Buffer to dissolve before use; then add 0.5 ml into 0.5 ml Assay Buffer, mix; the concentration will be 10 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 505 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For urine, serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Standard	Sample	Blank
Reaction Buffer	70 µl	70 µl	70 µl
Standard	20 µl	--	--
Sample	--	20 µl	--
Distilled water	--	--	20 µl
Enzyme	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, put it in the oven, 37 °C for 30 minutes, measured at 505 nm and record the absorbance.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Xanthine (μmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Xanthine (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$\text{Xanthine (μmol/10}^4\text{)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$\text{Xanthine (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 10 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

$C_{Standard}$: the standard concentration, 10 mmol/L = 10 μmol/ml;

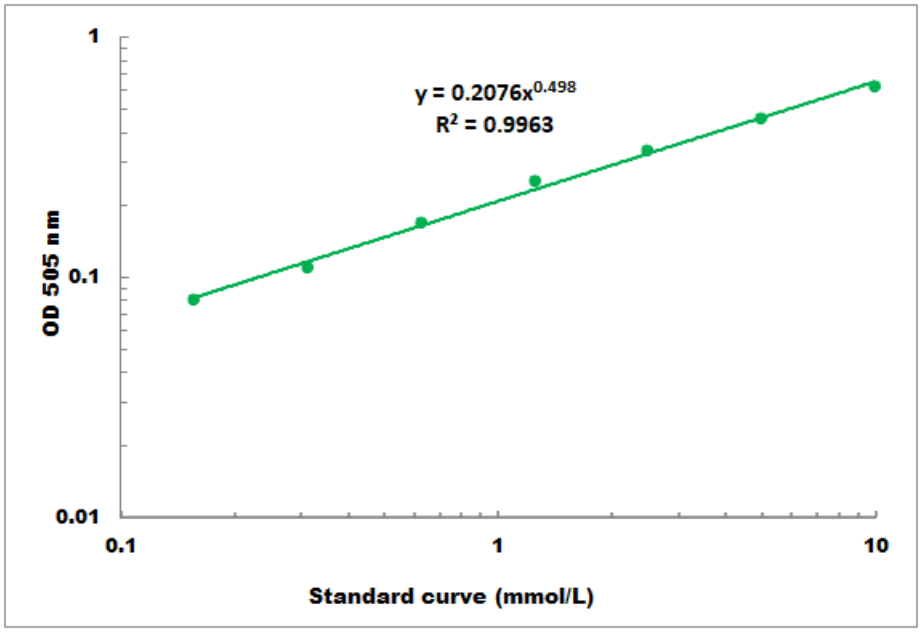
$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES