Xanthine/Hypoxanthine Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6818

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 .

1. 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 prog resses 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 st udies and for diagnostic and therapeutic monitoring.

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

2. Kit Components

Component	Volume	Storage
96-WellMicroplate	1 plate	-
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 mL x 1	4 °C
Enzyme	50 µlx 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	-

3. 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

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL Assay Buffer to generate 20 mmol/ L of standardstock solution, store at 4°C for 1-2 weeks or -20°C for 3 month safter reconstitution. Then dilute to10mmol/ Lstandard top solution by adding 0.5 mL stock solution into 0.5 mL Assay Buffer.Perform 2-fold serial dilutions of the top standard solution using Assay Buffer to make the standard curve.The concentration of standard curve could be 10/5/2.5/1.25/0.625/0.312 /0.156 mmol/L.

Enzyme: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use.Store at 4°C for 1 -2 days or -20°C for 3-4 weeks after reconstitution.

Dye Reagent: Add 10 mL Reaction Buffer to dissolve before use. Keep in dark and store at 4°C for 2-3 weeks or -20°C for 2 months.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. 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 20minutes , 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 8000 g 4°C for 2 0minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For urine, serum or plasma samples Detect directly.

6. Assay Procedure

Warm all reagents to room temperature before use . Add following reagents in to 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 recordthe absorbance.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

2.1According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \ (\mu mol/10^4)$$

2.2 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu mol/g)$$

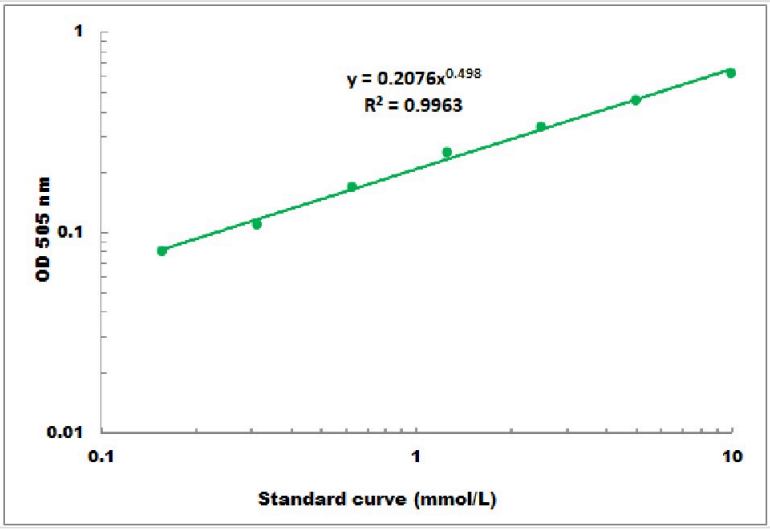
2.3According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \ (\mu mol/ml)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, mmol/L = μmol/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl
V_{Assay}	the volume of Assay Buffer, μl
W	the weight of sample, g
N	the quantity of cell or bacteria, 10^4

8. 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