Xanthine Oxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1056

(Version 1.3F)

Detection and Quantification of Xanthine Oxidase(XOD)Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

Xanthine Oxidase (XO) catalyzes the sequential oxidation of hypoxanthine to xanthine, and xanthine to uric acid and hydrogen peroxide. In humans and other primates, XO controls the final step of purine catabolism and is normally found in the liver and the intestinal mucosa. In rodents, XO is broadly expressed in most tissues. While XO activity is normally very low in blood, liver injury can result in the release of XO into blood. XO may contribute to the pathogenesis of gout and cardiovascular disease, and XO activity or expression may be upregulated in these conditions.

Xanthine Oxidase ActivityColorimetric Microplate Assay Kit is a sensitive assay for determining Xanthine Oxidaseactivity in various samples.The enzyme catalysed reaction product quinone can be measured at a colorimetric readout at 505 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-WellMicroplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	15 ml x 1	4 °C
Substrate	Powderx 1	4 °C
Substrate Diluent	1 ml x 1	4 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Standard (2.5mmoI/L)	1 ml x 1	4 °C, keep in dark
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 2.5/1.25/0.625/0.312/0.156/0.078/0.039mmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Substrate Diluentto dissolve before use.Prepare for immediate use or store at -20°C for 1-2 weeksafter reconstitution.

Dye Reagent: Add 10 ml Reaction Bufferto dissolve before use.Prepare for immediate use.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Bufferto dissolve before use, then add 0.1 ml into 0.9 ml Assay Buffer for detection.Store at -80°C for 1 month after reconstitution.

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

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Blank	Standard	Positive Control
Reaction Buffer	40 µl	40 µl	40 µl	40 µl
Substrate	10 µl	10 µl	10 µl	10 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Standard	--	--	50 µl	--
Distilled water	--	50 µl	--	--
Sample	50 µl	--	--	--
Positive Control	--	--	--	50 µl
Mix, put it in the oven,37 °C for 5 minutes, measured at 505 nm and recordthe absorbance.				

Note:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of XODactivity is defined as the enzyme generates 1μmolH_2O_2 per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(OD_{Sample} - OD_{Blank}) - \text{Intercept}}{\text{Slope} \times T} \times n(U/mL)$$

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

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times C_{Protein} \times T}(U/mg)$$

2.2According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay}) \times T}(U/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay}) \times T}(U/g)$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T}(U/mL)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Protein}$: the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

$C_{Standard}$: the concentration of standard, mmol/L= μmol/mL

$V_{Standard}$: the volume of standard in assay procedure, mL

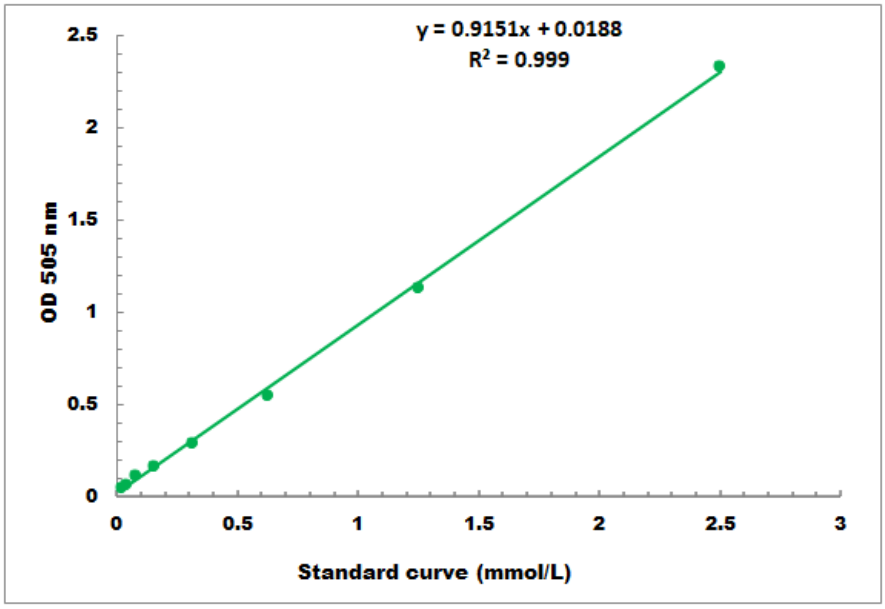
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

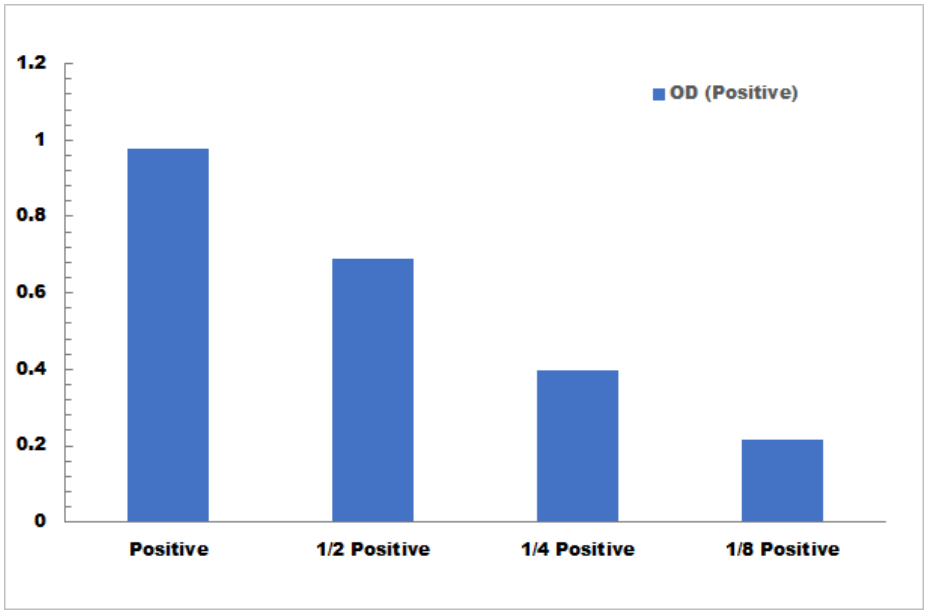
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 0.025 mmol/L-2.5 mmol/L



Positive Control reaction in 96-well plate assay with decreasing the concentration