Xanthine Oxidase Microplate Assay Kit

User Manual

Catalog # CAK1056

(Version 1.2F)

Detection and Quantification of Xanthine Oxidase (XOD) Activity 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 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 Microplate Assay Kit is a sensitive assay for determining Xanthine Oxidase activity in various samples. The enzyme catalysed reaction products quinone can be measured at a colorimetric readout at 505 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	15 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	1 ml x 1	4 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Standard (2.5 mmol/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	

Note:

Substrate: add 1 ml Substrate Diluent to dissolve before use.

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

Positive Control: add 1 ml Assay Buffer to dissolve before use, then add 0.1 ml into 0.9 ml Assay Buffer for detection.

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 serum or plasma samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the 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 record the absorbance.				

Note:

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

2) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

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

VI. CALCULATION

Unit Definition: One unit of XOD activity is defined as the enzyme generates 1 μmol H_2O_2 per minute.

1. According to the protein concentration of sample

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

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

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

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$XOD\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Protein}$: 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, 2.5 mmol/L = 2.5 μmol/ml;

$V_{Standard}$: the volume of the standard, 0.05 ml;

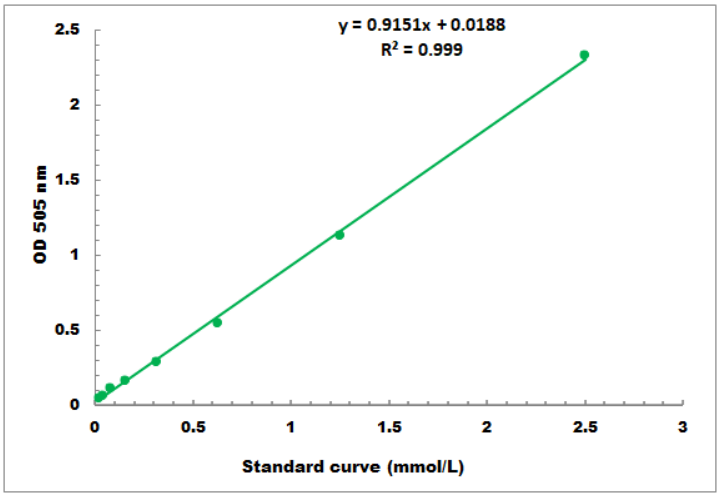
V_{Sample}: the volume of sample, 0.05 ml;

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

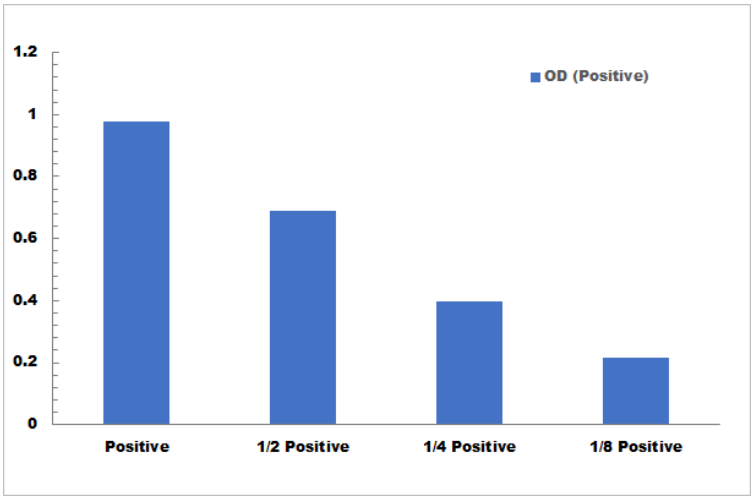
T: the reaction time, 5 minutes.

VII. 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

VIII. TECHNICAL SUPPORT

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

IX. NOTES