UricaseMicroplate Assay Kit

User Manual

Catalog # CAK1263

(Version 1.1B)

Detection and Quantification of Uricase(UO)Activity in Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

I. INTRODUCTION

Uricase (EC 1.7.3.3) is an enzyme involved in the uric acid metabolism. Uric acid is the end product of purine metabolism, and high levels of uric acid in blood causes gout. Uricase is present in a wide range of mammals but absent from human beings. Uricase Microplate Assay Kit provides a simple and direct procedure for measuring uricaseactivity in a variety of samples. The assay is initiated with the enzymatic catalysis of the uric acid by uricase. The enzyme catalysed reaction products quinone can be measured at a colorimetric readout at 550 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	1 ml x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent	Powder x 1	-20 °C, keep in dark
Standard (3 mmol/L)	1 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml Substrate Diluentto dissolve before use, mix.

Enzyme: add 1 ml Assay Bufferto dissolve before use, mix.

Dye Reagent: add 10 ml Distilled waterto dissolve before use, mix.

Positive Control: add 1 ml Assay Buffer to dissolve before use, mix.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 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 mlAssay buffer for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10000g 4°C for 10 minutes, 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 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagentsto room temperature before use.

Add following reagents intothe microplate:

Reagent	Standard	Blank	Sample	Control	Positive Control
Reaction Buffer	90 µl	90 µl	70 µl	70 µl	70 µl
Substrate	--	--	10 µl	10 µl	10 µl
Enzyme	--	--	10 µl	10 µl	10 µl
Mix, incubate at 37 °C for 5 minutes.					
Standard	10 µl	--	--	--	--
Distilled water	--	10 µl	--	10 µl	--
Sample	--	--	10 µl	--	--
Positive Control	--	--	--	--	10 µl
Dye Reagent	100 µl	100 µl	100 µl	100 µl	100 µl
Mix, put it in the oven,37 °C for 10 minutes, measured at 550 nm and recordthe 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 uricase is defined asthe enzyme generates 1 μmol of H2O2 per minute.

1. According to the protein concentration of sample

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

$$= 0.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 0.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 0.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

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

$$= 0.3 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the standard concentration, 3 mmol/L = 3μmol/ml;

W: the weight of sample, g;

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

V_{Sample}: the volume of sample, 0.01 ml;

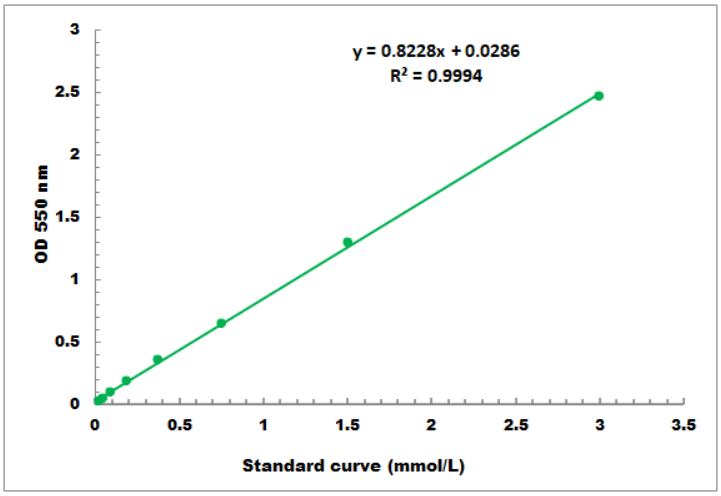
$V_{Standard}$: the volume of standard, 0.01 ml;

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

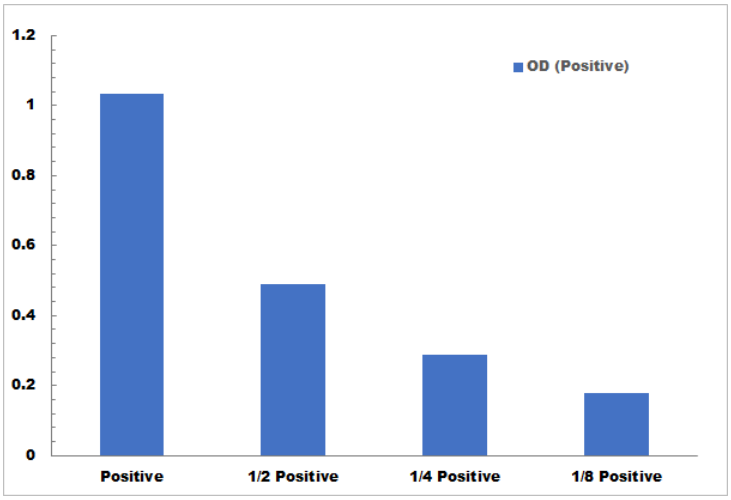
T: the reaction time, 10 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.03 mmol/L - 3 mmol/L



Positive Control reaction in 96-well plate assay with decreasing theconcentration

VIII. TECHNICAL SUPPORT

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

IX. NOTES