Urate Oxidase Assay Kit

ARG83430 Urate oxidase Assay Kit can be used to measure Urate oxidase in tissue extracts, cell lysate, cell culture media and other biological fluids.

Catalog number: ARG83430

Package: 96 wells

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INTRODUCTION

This enzyme was previously thought to be a copper protein, but it is now known that the enzymes from soy bean (Glycine max), the mould Aspergillus flavus and Bacillus subtilis contains no copper nor any other transition-metal ion. The 5-hydroxyisourate formed decomposes spontaneously to form allantoin and CO2, although there is an enzyme-catalysed pathway in which EC 3.5.2.17, hydroxyisourate hydrolase, catalyses the first step. The enzyme is different from EC 1.14.13.113 (FAD-dependent urate hydroxylase).

PRINCIPLE OF THE ASSAY

Urate oxidase Assay Kit determined Urate oxidaseactivity in tissue extracts, cell lysate, cell culture media and other biological fluids. The increase in absorbance at 550 nm is directly proportional to the Urate Oxidase.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard (3µmol/ml)	1 ml	4°C
Enzyme	1 vial (lyophilized)	-20°C
Assay Buffer	4 X 30 ml	4°C
Substrate	1 vial (lyophilized)	4°C
Substrate Diluent	1 ml	4°C
Reaction Buffer	10 ml	4°C
Reagent Dye	1 vial (lyophilized)	-20°C
Positive Control	1 vial (lyophilized)	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 550 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Enzyme Reagent Dye and Positive Control at -20°C, all other component at 4°C.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Avoid using reagents from different batches.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

For cell and bacteria -Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 106 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.

For tissue - Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

Note: For other liquid sample, it can be assayed directly.

REAGENT PREPARATION

- Substrate: Reconstitute the Substrate with 1 ml of Substrate Diluent. Allow the Substrate keep on bench for few minutes. Make sure the Substrate is dissolved completely and mixed thoroughly before use.

- Enzyme: Reconstitute the Enzyme with 1 ml of Assay Buffer. Allow the Enzyme keep on bench for few minutes. Make sure the Enzyme is dissolved completely and mixed thoroughly before use.

- **Reagent Dye:** Reconstitute the Reagent Dye with **1 ml** of **Distilled Water**. Allow the Reagent Dye keep on bench for few minutes. Make sure the Enzyme is dissolved completely and mixed thoroughly before use.

- **Positive Control:** Reconstitute the Positive Control with **1 ml** of **Assay Buffer**. Allow the Positive Control keep on bench for few minutes. Make sure the Positive Control is dissolved completely and mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **90 µl Reaction Buffer** into Standard wells, add **70 µl Reaction Buffer** into Sample, Control and Positive Control wells
2. Add **10 µl Substrate** into Sample, Control and Positive Control wells
3. Add **10 µl Enzyme** into Sample, Control and Positive Control wells
4. Mix well. Incubate at **37°C** for **5 min**
5. Add **10 µl Standard** into Standard wells.
6. Add **10 µl Distilled water** into Control wells.
7. Add **10 µl Sample** into Sample wells.
8. Add **10 µl Positive Control** into Positive Control wells.
9. Add **100 µl Reagent Dye** into Reagent Dye wells.
10. Mix well. Incubate at **37°C** for **10 min**
11. Read the OD at **550nm**

Summary of Urate oxidase Assay Procedure

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 well. Incubate at **37°C** for **5 min**					
Standard	10 µl	-	-	-	-
Distilled water	-	10 µl	-	10 µl	-
Sample	-	-	10 µl	-	-
Positive Control	-	-	-	-	10 µl
Reagent Dye	**100 µl**	**100 µl**	**100 µl**	**100 µl**	**100 µl**
Mix well. Incubate at **37°C** for **10 min** Read the OD at **550nm**					

CALCULATION OF RESULTS

1. Unit Definition: One unit Urate oxidaseactivity is defined as generates 1 µmol of H2O2 per minute in the reaction system.

2. Calculate the average absorbance values for each set of samples and control.

3. Calculation:

A. Definition:

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

$C_{Standard}$: the concentration of Standard, 3 µmol/ml;

V_{Sample}: the volume of reaction sample, 10 µl = 0.01 ml;

$V_{Standard}$: the volume of reaction Standard, 10 µl = 0.01 ml;

V_{assay}: the volume of Assay Buffer, 1000 µl = 1 ml;

W: the weight of sample, g;

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

T: the reaction time, 10 minutes.

B. Formula:

a). According to the protein concentration of sample

Urate oxidase activity (U/mg) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Control})] / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T)]$

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

b). According to the weight of sample

Urate oxidase activity (U/g) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{assay}) \times T)]$

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

c). According to the cell or bacteria

Urate oxidase activity (U/10^4) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (N \times V_{Sample} / V_{assay}) \times T)]$

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

d). According to the volume of sample

Urate oxidase activity (U/ml) =

$[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times T)]$

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