Xanthine Assay Kit

Xanthine Assay Kit can be used to measure Xanthine in serum and cell lysate samples.

Catalog number: ARG82197

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Xanthine is a purine base found in most human body tissues and fluids and in other organisms. Several stimulants are derived from xanthine, including caffeine and theobromine.

Xanthine is a product on the pathway of purine degradation e.g. from guanine by guanine deaminase, from hypoxanthine by xanthine oxidoreductase and also from xanthosine by purine nucleoside phosphorylase.

Xanthine is subsequently converted to uric acid by the action of the xanthine oxidase enzyme [Wikipedia: Xanthine]

PRINCIPLE OF THE ASSAY

This Xanthine Activity Assay Kit provides a simple, and rapid procedure for measuring Xanthine concentration in serum and cell lysate samples. In this assay, Xanthine is converted to uric acid by xanthine oxidase and H2O2 is released while reaction. The H2O2 is then reacting with HRP enzyme and the dye in the kit into a colored and fluorescent form. The color intensity at 570 nm or fluorescence intensity at λ ex/em = 530/585 nm is directly proportional to the Xanthine concentration in the sample. The concentration of Xanthine in the sample is then determined by comparing the signals of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Assay Buffer	10 ml (Ready to use)	-20°C
Xanthine Standard (2 mM)	1 ml	-20°C
Xanthine oxidase Enzyme	100 µl (Ready to use)	-20°C
HRP Enzyme	120 µl (Ready to use)	-20°C
Dye Reagent	120 µl (Ready to use)	-20°C

The kit is shipped on ice. Store all components at -20°C in dark. Shelf life of six months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 570 nm. Or fluorescence Microplate Reader capable of measuring fluorescence at λ ex/em = 530/585 nm.
- Flat bottomed 96-well microplate or Black flat bottomed 96-well microplate
- 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.
- Prior to assay, concentrations of protein, inhibitor, substrate and incubation time may need to be established for a given sample.
- Briefly spin down the reagents before use.
- It is recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use. During experiment, keep thawed Enzyme in a refrigerator or on ice.

SAMPLE COLLECTION & STORAGE INFORMATION

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

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot & store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Cell lysate- Homogenized 2 x10^6 of cells in 100 µL PBS. Then centrifuge at 14,000 rpm for 5 min. Use clear supernatant for assay. Collect Samples and assay immediately or aliquot & store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Standard:**

  For Colorimetric Procedure:

  - Dilute 2 mM Xanthine Standard standard solutions with deionized water to 2000 µM, 1200 µM and 600 µM as following table, and use deionized water serves as zero standard (0 µM, substrate blank).

  The example of the dilution of standards

Standard No.	Deionized water (µl)	2 mM Stock Standard (µl)	Standard Conc. µM
S1	0	100 µl	2000
S2	40	60 µl	1200
S3	70	30 µl	600
S0	100	0	0

  For fluorimetric Procedure:

  - For fluorimetric assays, the linear detection range is 3 to 250 µM Xanthine. Dilute the standards prepared in Colorimetric Procedure 1:10 with deionized water to yield standard concentration as 200 µM, 120 µM and 60 µM.

- **Working Reagent:**

  For each reaction combine the following (*Prepare before use)*:

  90 µL of Assay Buffer

  1 µL of Xanthine oxidase Enzyme

  1 µL of HRP Enzyme (vortex briefly before pipetting)

  1 µL Dye Reagent.

  It is recommended prepare freshly before use and prepare bulk working reagent using for once. Transfer 90 µl of Working Reagent to each sample

and standard well.

- **Assay buffer and enzymes:** Assay buffer and enzymes are ready to use, mix it well by vortex briefly before use.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use. During experiment, keep thawed Enzyme in a refrigerator or on ice.

For Colorimetric Procedure:

1. Add 10 µL of samples and standard (S0-S3, 0 µM, 2000 µM, 1200 µM and 600 µM) to separate wells in a 96 well plate.
2. Add 90 µL of the Working Reagent to each well.
3. Gently tap the plate to ensure thorough mixing. Incubate for 30 min at room temperature in dark.
4. Read the OD with a microplate reader at 570 nm (550 - 585 nm) immediately.

For Fluorimetric Procedure (black 96 well plate is used):

1. Add 10 µL of samples and standard (S0-S3, 0 µM, 200 µM, 120 µM and 60 µM) to separate wells in a 96 well plate.
2. Add 90 µL of the Working Reagent to each well.
3. Gently tap the plate to ensure thorough mixing. Incubate for 30 min at room temperature in dark.
4. Read fluorescence intensity at λ ex = 530 nm and λ em = 585 nm

immediately.

Summary:

	Assayed sample	Standard S0-S3
Sample	10 µl	
Standards		10 µl
Working Reagent	90 µl	90 µl
Mix well and incubate for 30 min at RT in dark.		
Read the OD with a microplate reader at 570 nm immediately. (Or read fluorescence intensity at λ ex/em = 530 / 585 nm immediately.)		

Note: The concentration of Standard S1-S3 are different between Colorimetric Procedure (2000 µM, 1200 µM, 600 µM) and Fluorimetric Procedure (200 µM, 120 µM, 60 µM). Please refer the detail at REAGENT PREPARATION section.

CALCULATION OF RESULTS

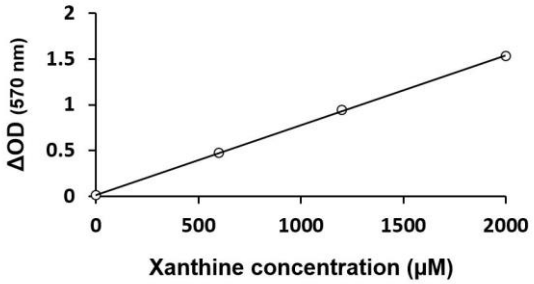
1. Subtract the blank value (S0) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the Xanthine concentration of the Samples as follows:

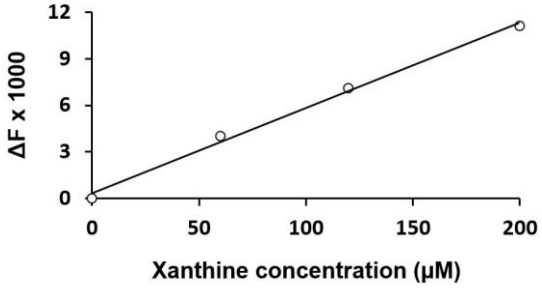
   [Xanthine] (µM)=

   $N \times [(R_{SAMPLE} - R_{BLANK}) / Slope\ (\mu M^{-1})]$

2. The R_{Sample} and R_{Blank} are OD or fluorescence readings of the Sample, and blank (S0). **N** is the sample dilution factor.
3. If the calculated Xanthine concentration is >2000 µM for the colorimetric assay, or >200 µM for the fluorimetric assay, dilute sample in deionized water and repeat assay. Multiply result by the dilution factor N.

EXAMPLE OF ASSAY





QUALITY ASSURANCE

Sensitivity

Linear detection range:

Colorimetric assays: 10 to 2000 µM

Fluorimetric assays: 3 to 200 µM

The minimum detectable dose (MDD) of Xanthine was:

Colorimetric assays: 10 µM

Fluorimetric assays: 3 µM