USER GUIDE

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**Version 2.0**

# 黄嘌呤检测试剂盒

# Xanthine Assay Kit

**Do not eat** **Store at -20° C & in the dark.**

**Cat.No.**MXA4685 **Size :** 100 tests

**Technical literature is available at:** www.mesgenbio.com
**E-mail MesGen Technical Services if you have questions on use of this system:** tech@mesgenbio.com

## Description

Xanthine is a purine base that can be found in most animal tissues and fluids. It is a product in the purine degradation pathway, produced by guanine deaminase from guanine, and by xanthine oxidoreductase from hypoxanthine. Xanthine is degraded to uric acid by xanthine oxidase. Clinically, xanthine and its derivatives act on sleep-inducing adenosine receptors as antagonists. Simple, direct and high-throughput assays for measuring xanthine find wide applications in research and drug discovery. MesGen Biotechnology xanthine assay kit uses a single Working Reagent that combines the xanthine oxidase reaction and color reaction in one step. The change in color intensity of the reaction product at 570 nm or fluorescence intensity at lex/em = 530/585 nm is directly proportional to xanthine concentration in the sample.

## Application

**Direct Assays:** xanthine concentration in cell lysate, serum, and other biological samples.

**Drug Discovery/Pharmacology:** effects of drugs on xanthine (purine) metabolism.

## Key features

**Sensitive and accurate.** Use as little as 10 µL samples. Linear detection range in 96-well plate for 30 minute incubation: 0.01 to 2 mM xanthine for colorimetric assays and 3 to 200 µM for fluorimetric assays.

**Simple and convenient.** The procedure involves addition of a single working reagent and incubation for 30 min at room temperature.

**Fast and high-throughput.** Assays using 96-well plates and liquid handling system could allow simultaneous processing tens of thousands of samples per day.

## Kit contents

**Reagent A :** 8 mL **Reagent B :** 1 mL
**Reagent C :** 1 mL **Reagent D :** 100 µL
**Standard:** 1 mL 2mM Xanthine

## Colorimetric Procedures

Samples can be analyzed immediately after collection, or stored in aliquots at -20°C. Avoid repeated freeze-thaw cycles. If particulates are present, centrifuge sample and use clear supernatant for assay.

1. Equilibrate all components to room temperature. During experiment, keep thawed Enzyme in a refrigerator or on ice.

2. *Standard Curve*. Prepare standards as shown in the Table below.

| No. | Premix + $H_2O$ | Vol (µL) | Xanthine (mM) |
|---|---|---|---|
| **1** | 100 µL + 0 µL | 100 | 2.0 |
| **2** | 80 µL + 20 µL | 100 | 1.6 |
| **3** | 60 µL + 40 µL | 100 | 1.2 |
| **4** | 40 µL + 60 µL | 100 | 0.8 |
| **5** | 20 µL + 80 µL | 100 | 0.4 |
| **6** | 0 µL + 100 µL | 100 | 0 |

Transfer 10 µL standards and samples into separate wells.

3. *Working Reagent*. Prepare bulk working reagent by mixing 90 µL Reagent A, 10 µL Reagent B, 10 µL Reagent C,and 1 µL Reagent D per reaction well in a clean tube. Transfer 100 µL Working Reagent into each reaction well. Tap plate to mix.

4. Incubate 30 min at room temperature, and then read optical density at 570 nm (550-585 nm) .

## Fluorimetric Procedures

For fluorimetric assays, the linear detection range is 3 to 250 µM xanthine.Dilute the standards from Colorimetric Procedure 10X with $dH_2O$ to obtain standards at 200, 120, 60 and 0 µM Xanthine.

Transfer 10 µL standards and 10 µL samples into separate wells of a black 96-well plate. Add 100 µL Working Reagent (see Colorimetric Procedure), tap plate to mix. Incubate 30 min at room temperature, and then read fluorescence at lex/em = 530/585 nm.

**Calculation**

Subtract blank OD30 or F30 (water, #4) from all standards and samples OD30 or F30 values and plot the DOD or ΔF against standard concentrations. Calculate the concentration using the equation below:

$$[\text{Xanthine}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

Where $R_{Sample}$ and $R_{Blank}$ are the optical density or fluorescent values of the sample and blank, respectively. Slope is the slope of the standard curve and n is the dilution factor.

Notes: If the calculated sample xanthine concentration is higher than 2 mM in colorimetric assay or 200 μM in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor (n).

**Precautions**

Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

**Storage conditions**

The kit is shipped on ice. Store all reagents at -20°C.

**Shelf life**

6 months after receipt.

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