

For Research Use Only. Not For Use In Diagnostic Procedures

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 $\lambda_{ex}/\lambda_{em} = 530/585$ 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 μ L samples. Linear detection range in 96-well plate for 10-15 minute incubation: 2 μ M to 1 mM xanthine for colorimetric assays and 0.5 to 200 μ M for fluorimetric assays.

Simple and convenient. The procedure involves addition of a single working reagent and incubation for 20 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 : 5 mL Reagent B : 5 mL

Reagent C : 200 μ L

Standard: 0.5 mL 5 mM 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 ₂ O	Vol (μ L)	Xanthine (μ M)
1	20 μ L + 80 μ L	100	1000
2	10 μ L + 90 μ L	100	500
3	5 μ L + 95 μ L	100	250
4	1 μ L + 99 μ L	100	50
5	0.2 μ L + 99.8 μ L	100	10
6	0 μ L + 100 μ L	100	0

Transfer 10 μ L standards and samples into separate wells.

3. *Working Reagent.* Prepare bulk working reagent by mixing 50 μ L Reagent A, 50 μ L Reagent B and 2 μ L Reagent C per reaction well in a clean tube. Transfer 100 μ L Working Reagent into each reaction well. Tap plate to mix.

4. Incubate 10-15 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 0.5 to 200 μ M xanthine. Dilute the standards from Colorimetric Procedure with dH₂O to obtain standards at 200, 100, 60, 10, 1 and 0 μ M Xanthine.

Transfer 10 μ L standards and 10 μ L samples into separate wells of a black 96-well plate. Add 100 μ L Working Reagent (see Colorimetric Procedure), tap plate to mix. Incubate 10-15 min at room temperature, and then read fluorescence at $\lambda_{ex}/\lambda_{em} = 530/585$ 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:

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

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 1 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

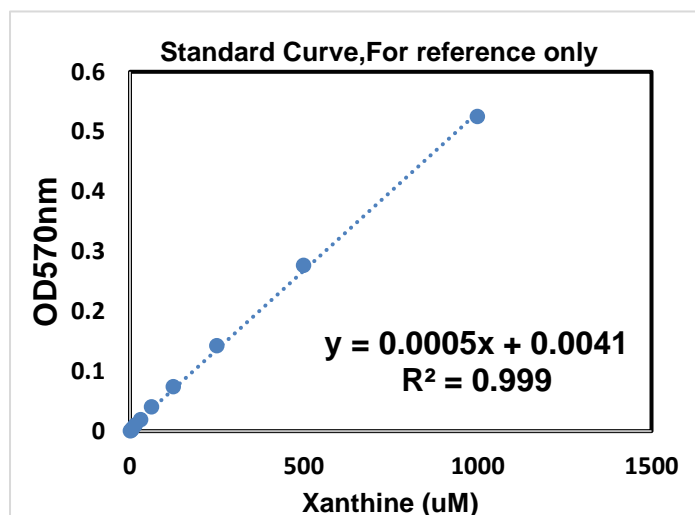
Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

Storage conditions

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

Shelf life

6 months after receipt.



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