EnzyChrom™ Xanthine Assay Kit (EXAN-100)

Quantitative Colorimetric/Fluorimetric Xanthine Concentration Determination

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. BioAssay Systems' 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 $\lambda_{ex/em}$ = 530/585 nm is directly proportional to xanthine concentration in the sample.

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.

APPLICATIONS

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

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

KIT CONTENTS (100 tests in 96-well plates)

Assay Buffer: 10 mL
Standard: 1 mL 2 mM Xanthine
XO Enzyme: 100 µL
HRP Enzyme: 120 µL
Dye Reagent: 120 µL

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.

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.

COLORIMETRIC PROCEDURE

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	Standard + H_2O	Vol (µL)	Xanthine (mM)
1	100 µL + 0 µL	100	2
2	60 µL + 40 µL	100	1.2
3	30 µL + 70 µL	100	0.6
4	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 Assay Buffer, 1 µL XO Enzyme, 1 µL HRP Enzyme (*vortex briefly before pipetting*), and 1 µL Dye Reagent per reaction well in a clean tube. Transfer 90 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) (OD_{30}).

FLUORIMETRIC PROCEDURE

For fluorimetric assays, the linear detection range is 3 to 250 µM xanthine. Dilute the standards from *Colorimetric Procedure* 10× 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 90 µL Working Reagent (see *Colorimetric Procedure*), tap plate to mix.

Incubate 30 min at room temperature, and then read fluorescence at $\lambda_{ex/em}$ = 530/585 nm (F_{30}).

CALCULATION

Subtract blank OD_{30} or F_{30} (water, #4) from all standards and samples OD_{30} or F_{30} values and plot the ΔOD or ΔF against standard concentrations. Calculate the concentration using the equation below:

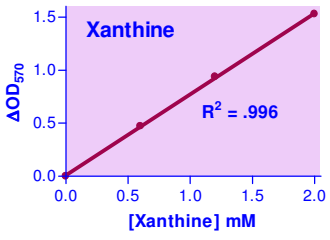
$$[\text{Xanthine}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

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.

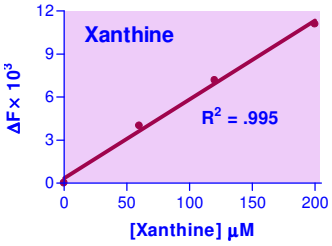
Notes: If the calculated sample xanthine concentration is higher than 2 mM in colorimetric assay or 200 µM in fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor (*n*).

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), black 96-well plates (e.g. Greiner Bio-One, cat# 655900) and plate reader capable of either measuring absorbance between 550-585 nm or fluorescence intensity at $\lambda_{ex/em}$ = 530/585 nm.



96-well colorimetric assay



96-well fluorimetric standard

LITERATURE

1. Ukena D, Schudt C, Sybrecht GW (1993). Adenosine receptor-blocking xanthines as inhibitors of phosphodiesterase isozymes. Biochem. Pharm. 45(4): 847–51.

2. Daly JW, Hide I, Müller CE, Shamim M (1991). Caffeine analogs: structure activity relationships at adenosine receptors. Pharmacology. 42(6): 309–21.

3. González MP, Terán C, Teijeira M (2008). Search for new antagonist ligands for adenosine receptors from QSAR point of view. How close are we?. Medicinal Research Reviews. 28(3): 329–71.