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Product Information

Xanthine/Hypoxanthine Assay Kit

Catalog Number **MAK186**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Xanthine is a purine base present in body fluids, tissues, and plants cells. It is also found in urinary calculi. Xanthine is formed by the oxidation of hypoxanthine during the degradation of adenosine monophosphate (AMP) to uric acid. Xanthine/ hypoxanthine measurements in plasma and urine have been used to monitor allopurinol therapy.[1] Cerebrospinal fluid (CSF) xanthine/hypoxanthine levels have been used as therapeutic guides and disease progression markers in hydrocephalus.[2]

The Xanthine/Hypoxanthine Assay Kit is a highly sensitive assay for determining xanthine/hypoxanthine levels (ranging from 4–20 nmole/well for the colorimetric assay and 40–200 pmole/well for the fluorometric assay) in a variety of biological samples. Xanthine/hypoxanthine concentration is determined by an enzymatic assay, which results in a colorimetric (570 nm) or fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the xanthine/hypoxanthine present.
Note: The fluorometric assay is ~10 times more sensitive than the colorimetric assay.

Components

The kit is sufficient for 100 assays in 96 well plates.

Xanthine Assay Buffer Catalog Number MAK186A	25 mL
Peroxidase Substrate Catalog Number MAK186B	0.2 mL
Xanthine Enzyme Mix Catalog Number MAK186C	1 vl
Developer Catalog Number MAK186D	1 vl
Xanthine Standard Catalog Number MAK186E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays and black plates for fluorometric assays.
- Spectrophotometric or fluorometric multiwell plate reader
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter (optional for protein-containing samples)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Xanthine Assay Buffer – Allow buffer to come to room temperature before use.

Peroxidase Substrate – Ready to use. Store at –20 °C.

Xanthine Enzyme Mix and Developer – Reconstitute each in 220 µL of Xanthine Assay Buffer Mix. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Xanthine Standard – Reconstitute in 500 µL of water to generate 2 mM (2 nmole/µL) Xanthine Standard Solution. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Xanthine Standards for Colorimetric Detection
Add 0, 2, 4, 6, 8, and 10 µL of the 2 mM (2 nmole/µL) Xanthine Standard Solution into a 96 well plate in duplicate, generating 0 (blank), 4, 8, 12, 16, and 20 nmole/well standards. Add Xanthine Assay Buffer to each well to bring the volume to 50 µL.

Xanthine Standards for Fluorometric Detection
Dilute 10 µL of the 2 mM (2 nmole/µL) Xanthine Standard Solution with 990 µL of water to generate 0.02 mM (20 pmole/µL) Xanthine Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of 0.02 mM (20 pmole/µL) Xanthine Standard Solution into a 96 well plate generating 0, 40, 80, 120, 160, and 200 pmole/well standards. Add Xanthine Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid samples can be assayed directly.

Tissue (10 mg) or cells (1×10^6) can be homogenized in 100 µL of ice-cold Xanthine Assay Buffer for 10 minutes on ice. Centrifuge the samples at 12,000 rpm for 5 minutes. Collect the supernatant.

Enzymes in samples may interfere with the assay. To remove enzymes from samples, deproteinize using a 10 kDa MWCO spin filter.

To correct for background in samples, include a Sample Blank by omitting the Xanthine Enzyme Mix. The Sample Blank readings can then be subtracted from the sample readings.

For unknown samples, it is suggested to test several sample volumes to ensure the readings are within the linear range of the standard curve.

Add 1–50 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with Xanthine Assay Buffer.

Assay Reaction
1. Set up Reaction Mixes according to the schemes in Table 1 or Table 2. 50 µL of Reaction Mix is required for each reaction (well).

Table 1.
Colorimetric Reaction Mixes

Reagent	Samples and Standards	Sample Blank
Xanthine Assay Buffer	44 µL	46 µL
Xanthine Enzyme Mix	2 µL	–
Developer	2 µL	2 µL
Peroxidase Substrate	2 µL	2 µL

Table 2.
Fluorometric Reaction Mixes

Reagent	Samples and Standards	Sample Blank
Xanthine Assay Buffer	45.8 µL	47.8 µL
Xanthine Enzyme Mix	2 µL	–
Developer	2 µL	2 µL
Peroxidase Substrate	0.2 µL	0.2 µL

Note: The fluorometric assay is ~10 times more sensitive than the colorimetric assay.

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the absorbance at 570 nm (A_{570}) or the fluorescence (FLU, λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (blank) Xanthine Standard. Correct for the background by subtracting the blank standard value from all readings. Background values can be significant and must be subtracted from all readings. Use the values (A_{570} or FLU) obtained from the appropriate Xanthine Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the Sample Blank value from the sample reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of xanthine or hypoxanthine present in the sample from the standard curve.

Concentration of Xanthine/Hypoxanthine

$$S_a/S_v = C$$

where:

S_a = Amount of Xanthine/Hypoxanthine in unknown sample well (nmole or pmole) from standard curve

S_v = Sample volume (μL) added into the well.

C = Concentration of Xanthine/Hypoxanthine in sample (nmole/μL or pmole/μL)

Xanthine molecular weight is 152.11 g/mole.

Hypoxanthine molecular weight is 136.11 g/mole.

Sample Calculation

Amount of Xanthine (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of Xanthine in sample:

$$5.84 \text{ nmole}/50 \text{ μL} = 0.1168 \text{ nmole/μL}.$$

$$0.1168 \text{ nmole/μL} \times 152.11 \text{ ng/nmole} = 17.77 \text{ ng/μL}.$$

References

1. Miyazaki, H. et al., Simultaneous determination of plasma and urinary uric acid, xanthine, hypoxanthine, allopurinol, oxipurinol, orotic acid, orotidine and creatinine by high-performance liquid chromatography. J. Chromatogr., **274(75)**, 75–85 (1983).
2. Castro-Gago, M. et al., The concentrations of xanthine and hypoxanthine in cerebrospinal fluid as therapeutic guides in hydrocephalus. Childs Nerv. Syst., **2(3)**, 109–111 (1986).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates.
Samples with erratic readings	Samples prepared in different buffer	Use the Xanthine Assay Buffer
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mixes before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range