Amplite™ Fluorimetric Xanthine Assay Kit

Catalog number: 13843
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red Substrate (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	20 mL
Component C: Horseradish Peroxidase (lyophilized)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component D: Xanthine Standard	Freeze (<-15 °C), Minimize light exposure	100 µL (20 mM)
Component E: Xanthine Oxidase (lyophilized)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component F: DMSO	Freeze (<-15 °C)	1 vial (100 µL)

OVERVIEW

Xanthine is a purine base found in most human body tissues and fluids. A number of stimulants are derived from xanthine, including caffeine, aminophylline, IBMX, paraxanthine, pentoxifylline, theobromine, and theophylline, which can stimulate heart rate, force of contraction, cardiac arrhythmias at high concentrations. Therefore, detection of Xanthine alteration in biological samples is important for disease diagnosis and therapy monitoring. Amplite™ Fluorimetric Xanthine Assay Kit provides a quick and ultrasensitive method for the measurement of xanthine. It can be performed in a convenient 96-well or 384-well microtiter-plate format. Xanthine is oxidized to uric acid in the presence of xanthine oxidase to release hydrogen peroxide, which can be specifically measured with Amplite™ Red by a fluorescence microplate reader. With Amplite™ Fluorimetric Xanthine Assay Kit, as low as 0.4 µM xanthine was detected in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. Prepare xanthine standards or test samples (50 µL)
2. Add xanthine working solution (50 µL)
3. Incubate at room temperature for 30 - 60 min
4. Read fluorescence intensity at Ex/Em = 540/590 nm

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Amplite™ Red substrate stock solution (250X):
Add 40 µL of DMSO (Component F) into the vial of Amplite™ Red substrate (Component A).

Note Amplite™ Red substrate is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The assay should be performed at pH 7 - 8 (pH 7.4 is recommended) as Amplite™ Red is unstable at pH > 8.5.

2. HRP stock solution (500X):
Add 100 µL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).

3. Xanthine oxidase (XO) stock solution (100X):
Add 100 µL of Assay Buffer (Component B) into the vial of Xanthine Oxidase (Component E) to make Xanthine Oxidase (XO) stock solution (100X).

PREPARATION OF STANDARD SOLUTION

Xanthine standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13843

Add 5 µL of Xanthine Standard (Component D) into 995 µL of Assay Buffer (Component B) to get 100 µM xanthine standard solution. Take 200 µL of 100 µM xanthine standard solution to perform 1:3 serial dilutions to get serially diluted xanthine standards (X1 - X7).

PREPARATION OF WORKING SOLUTION

Add 20 µL of Amplite™ Red Substrate stock solution (250X), 10 µL of HRP stock solution (500X), and 50 µL of Xanthine Oxidase stock solution (100X) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.08 mL.

Note Avoid direct exposure to light and use promptly.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Xanthine standards and test samples in a black wall/solid bottom 96-well microplate. X = xanthine standard (X1 - X7, 0.137 to 100 µM); BL = blank control; TS = test sample.

BL	BL	TS	TS
X1	X1	...	...
X2	X2	...	...
X3	X3		
X4	X4		
X5	X5		
X6	X6		
X7	X7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
X1 - X7	50 µL	Serial Dilution (0.137 to 100 µM)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	Test Sample

1. Prepare xanthine standards (X), blank controls (BL), and test samples (TS)

according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of xanthine working solution into each well of the xanthine standards, blank control, and test samples to make the total xanthine assay volume of 100 µL/well. For a 384-well plate, add 25 µL of working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.

4. Monitor the fluorescence increase with with a fluorescence plate reader at Excitation = 530 - 570 nm (optimal at 540 nm), Emission = 590 - 600 nm (optimal at 590 nm), cutoff = 570 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Xanthine samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

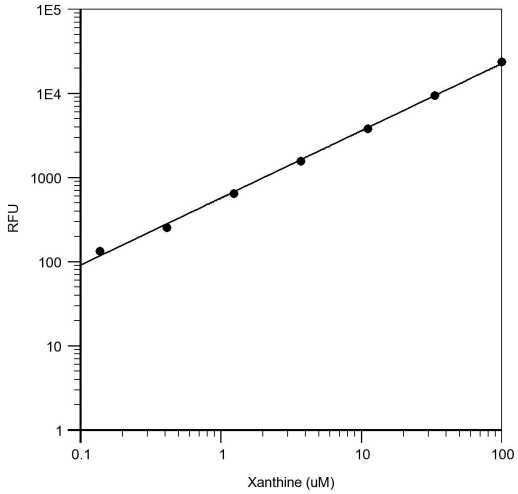

Figure 1. Xanthine dose response was measured with Amplite™ Fluorimetric Xanthine Assay Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices).

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