Amplite™ Fluorimetric Xanthine Oxidase Assay Kit *Red Fluorescence*

Catalog number: 11304
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	20 mL
Component C: Horseradish Peroxidase	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized)
Component D: Xanthine	Freeze (<-15 °C), Minimize light exposure	100 µL (100 X)
Component E: Xanthine Oxidase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (200 mU, lyophilized)
Component F: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

Xanthine oxidase (XO) is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. It plays an important role in the catabolism of purines. Xanthine oxidase is normally found in liver and jejunum. During severe liver damage, xanthine oxidase is released into blood, so a blood assay for XO is a way to determine if liver damage has happened. Xanthinuria is a rare genetic disorder where the lack of xanthine oxidase leads to high concentration of xanthine in blood and can cause health problems such as renal failure. The Amplite™ Fluorimetric Xanthine Oxidase Assay Kit provides a quick and ultrasensitive method for the measurement of xanthine oxidase activities. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. In the assay, xanthine oxidase catalyzes the oxidation of purine bases, hypoxanthine or xanthine to uric acid and superoxide , which spontaneously degrades to hydrogen peroxide (H2O2). The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The fluorescent signal can be easily read by either a fluorescence microplate reader or an absorbance microplate reader. With the Amplite™ Xanthine Oxidase Assay Kit, we have detected as little as 0.15 mU/mL xanthine oxidase in a 100 µL reaction volume.

AT A GLANCE

Protocol summary

1. XO standards or test samples (50 µL)
2. Add XO working solution (50 µL)
3. Incubate at room temperature for 15 - 30 minutes
4. Read fluorescence intensity at Ex/Em = 540/590 nm (cut off 570 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540nm
Emission:	590nm
Cutoff:	570nm
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 stock solution (250X):
Add 40 µL of DMSO (Component F) into the vial of Amplite™ Red substrate (Component A). The stock solution should be used promptly.

Note The 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 Amplite™ Red substrate is also unstable at high pH (>8.5). Therefore, the reaction should be performed at pH = 7 – 8. The provided assay buffer, pH = 7.4, is recommended.

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) standard solution (1 U/mL)
Add 200 µL of Assay Buffer (Component B) into the vial of Xanthine Oxidase Standard (Component E).

PREPARATION OF STANDARD SOLUTION

XO standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11304

Add 10 µL of 1 U/mL XO stanndard solution into 990 µL of Assay Buffer (Component B) to make 10 mU/mL XO standard solution (XO7). Perform 1:3 serial dilutions to get remaining serially diluted XO standards (XO6-XO1).

PREPARATION OF WORKING SOLUTION

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

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of XO standards and test samples in a solid black 96-well microplate.

BL	BL	TS	TS
XO1	XO1	...	...
XO2	XO2	...	...
XO3	XO3		
XO4	XO4		
XO5	XO5		
XO6	XO6		
XO7	XO7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
XO1 - XO7	50 µL	serial dilution (0.01 to 10 mU/mL)

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BL	50 µL	Assay Buffer (Component B)
TS	50 µL	sample

1. Prepare XO standards (XO), blank controls (BL), and test samples (TS) into a solid black 96-well microplate 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 XO working solution into each well of the XO standards, blank control, and test samples to make the total XO assay volume of 100 µL/well. For a 384-well plate, add 25 µL of XO working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction for 15 to 30 minutes at room temperature, protected from light.

4. Monitor the fluorescence intensity with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 590 - 600 nm (optimal Ex/Em = 540/590 nm), cut off = 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 Oxidase 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

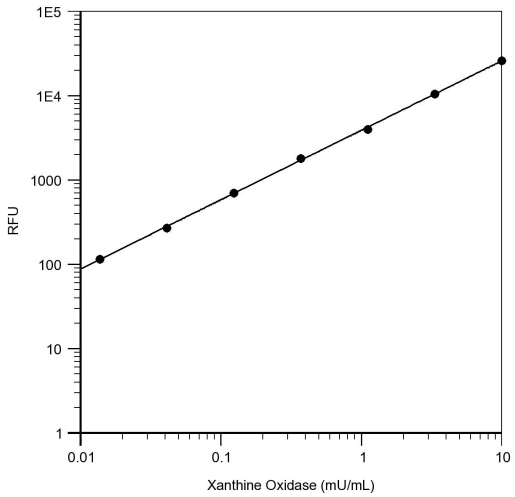

Figure 1. Xanthine oxidase dose response was measured with Amplite™ Fluorimetric Xanthine Oxidase Assay Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER