Amplite™ Colorimetric Xanthine Oxidase Assay Kit

Catalog number: 11307
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)	1 bottle (20 mL)
Component C: Horseradish Peroxidase	Freeze (< -15 °C), Minimize light exposure	1 vial (lyophilized)
Component D: Xanthine	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL, 100X)
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™ Colorimetric 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 color signal can be easily read at ~570 nm with an absorbance microplate reader. With the Amplite™ Colorimetric Xanthine Oxidase Assay Kit, we have detected as little as 0.3 mU/mL xanthine oxidase in a 100 µL reaction volume.

AT A GLANCE

Protocol Summary

1. Prepare and add XO standards and/or test samples (50 µL)
2. Prepare and add XO Assay working solution (50 µL)
3. Incubate at room temperature for 30-60 minutes
4. Read absorbance increase at OD ratio of 570/610 nm

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

KEY PARAMETERS

Absorbance microplate reader

Absorbance	570/610 nm
Recommended plate	Clear bottom

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 HRP (Component C).

Note The unused HRP stock solution (500X) should be divided into single use aliquots and stored them at -20 ° C.

3. Xanthine Oxidase (XO) stock solution
Add 200 µL of Assay Buffer (Component B) into the vial of Xanthine Oxidase Standard (Component E).

Note The unused XO stock solution should be divided into single use aliquots and stored at -20 ºC.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11307

Xanthine Oxidase standard
Add 10 µL of 1 U/mL XO stock solution into 990 µL of Assay Buffer (Component B) to make 10 mU/mL XO standard solution.Perform 1:3 serial dilutions to get approximately 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 mU/mL serially diluted XO standards.

PREPARATION OF WORKING SOLUTION

Table 1. XO Assay working solution for one clear bottom 96-well microplate (2X)

Components	Volume
Amplite&trade Red Stock Solution (250x)	20 µL
HRP Stock Solution (500X)	10 µL
Xanthine (100X)	50 µL
Assay Buffer	5 mL
Total volume	**5.08 mL**

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of Xanthine Oxidase standards and test samples in a clear bottom 96-well microplate. XOS = Xanthine Oxidase standard (XOS1-XOS7); BL = blank control; TS = test sample.

BL	BL	TS	TS
XOS1	XOS1	...	...
XOS2	XOS2	...	...
XOS3	XOS3		
XOS4	XOS4		
XOS5	XOS5		
XOS6	XOS6		
XOS7	XOS7		

Table 2. Reagent composition for each well

Well	Volume	Reagent
XOS1 - XOS7	50 µL	Serial Dilutions (0.01 to 10 µM)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	test sample

Note The xanthine oxidase standards are for positive control only, and should not be relied on as a quantitation standard for enzyme activity.

1. Add XO standards and XO containing test samples into a white clear bottom microplate as described in Tables 1 and 2.

2. Add 50 µL of XO Assay working solution into each well of XO standard, blank control, and test samples (Table 1) to make the total XO assay volume of 100 µL/well.

   Note For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.

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

4. Monitor signal intensity with an absorbance plate reader at OD ratio of 570/610 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Abs 570/Abs 610) 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

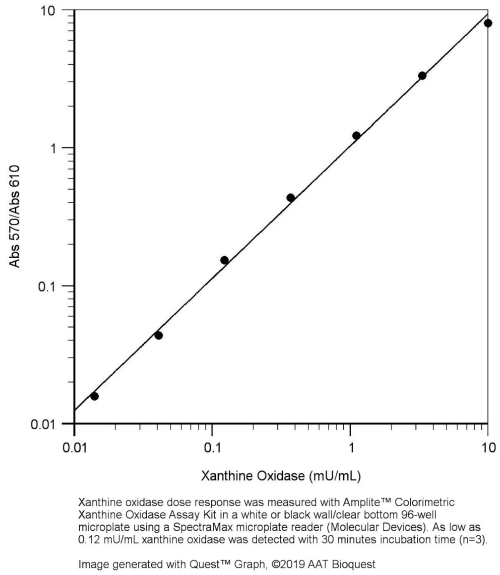

Figure 1. Xanthine oxidase dose response was measured with Amplite™ Colorimetric Xanthine Oxidase Assay Kit in a white or black wall/clear bottom 96-well microplate using a SpectraMax microplate reader (Molecular Devices). As low as 0.12 mU/mL xanthine oxidase was detected with 30 minutes incubation time (n=3).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.