Amplite™ Fluorimetric Xanthine Oxidase Assay Kit

Red Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 11304 (200 assays)	Keep at -20 ºC and protect from light	Fluorescence microplate readers

Introduction

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 the liver and jejunum. During severe liver damage, xanthine oxidase is released into the 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. Inhibition of xanthine oxidase has been proposed as a mechanism for improving cardiovascular health.

The Amplite™ Fluorimetric Xanthine Oxidase Assay Kit provides a quick and ultrasensitive method for the measurement of xanthine oxidase activity. Xanthine oxidase catalyzes the oxidation of purine bases, hypoxanthine or xanthine, to uric acid and superoxide, the superoxide spontaneously degrades to hydrogen peroxide (H_2O_2), which can be specifically measured by our Amplite™ Red substrate in a dual mode. The signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540 nm/590 nm or an absorbance microplate reader with the OD ratio at the wavelength of 570 nm to 610 nm. With the Amplite™ Fluorimetric Xanthine Oxidase Assay Kit, we have detected as little as 14 µU/mL xanthine oxidase in a 100 µL reaction volume.

Kit Components

Components	Amount
Component A: Amplite™ Red (light sensitive)	1 vial
Component B: Assay Buffer	20 mL
Component C: Horseradish Peroxidase	1 vial (lyophilized)
Component D: Xanthine	100 µL (100 X)
Component E: Xanthine Oxidase Standard	1 vial (200 mU, lyophilized)
Component F: DMSO	1 vial (200 µL)

Protocol for one 96-well plate

Brief Summary

XO standards or test samples (50 µL)→ Add XO assay mixture (50 µL)→ Incubate at room temperature for 15-30 minutes → Read fluorescence intensity at Ex/Em = 540/590 nm (cut off 570 nm)

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

1. Prepare stock solutions:

1.1 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. Any remaining solution should be aliquoted and frozen at -20 ºC.
Note 1: Avoid repeated freeze-thaw cycles.
Note 2: 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.

1.2 HRP stock solution (500X): Add 100 µL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).
Note: The unused HRP solution should be divided into single use aliquots and stored at -20 ºC.

1.3 1 U/mL 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.

2. Prepare assay mixture:

Prepare assay mixture according to Table 1 and protect from light.

Ordering:sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support:support@aatbio.com; Tel: 408-733-1055

Table 1. Assay mixture for one 96-well plate:

Components	**Volume**
Amplite™ Red Stock Solution (250X, from Step 1.1)	20 µL
HRP (500X, from Step 1.2)	10 µL
Xanthine (100X, Component D)	50 µL
Assay Buffer (Component B)	5 mL
Total volume	5.08 mL

3. Prepare serially diluted XO standards (0 to 10 mU/mL):

3.1 Add 10 µL of 1 U/mL XO stock solution (from Step 1.3) into 990 µL of Assay Buffer (Component B) to make 10 mU/mL XO standard solution.

3.2 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.

3.3 Add XO standards and XO-containing samples into a solid black 96-well microplate as in Tables 2 and 3.

Table 2. 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										

Note: XO =Xanthine oxidase standards, BL=Blank control, TS = test samples.

Table 3. Reagent composition for each well

XO Standard	**Blank Control**	**Test Sample**
Serial Dilutions* (50 µL)	Assay Buffer (Component B): 50 µL	50 µL

Note: Add the serially diluted xanthine oxidase standards from 14 µU/mL to 10 mU/mL into each well from XO1 to XO7 in duplicate.

4. Run XO assay:

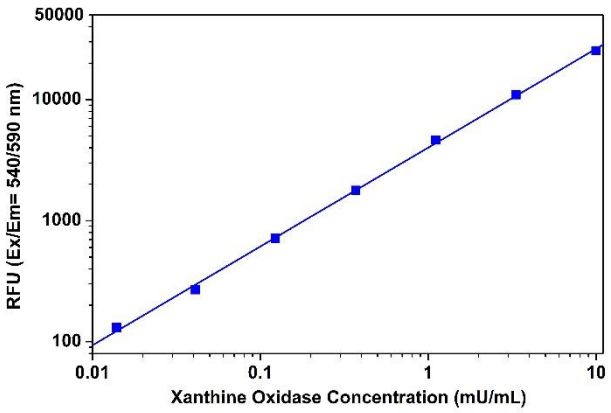
4.1 Add 50 µL of assay mixture (from Step 2) into each well of the XO standards, blank control, and test samples (see Step 3, Table 2) to make the total XO assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay mixture into each well.

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

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

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with xanthine oxidase reactions. The typical data are shown in Figure 1.



Note: High concentration of XO and longer incubation time may cause reduced fluorescence signal due to the over oxidation of Amplite™ Red substrate (to a non-fluorescent product).

Figure 1. Xanthine oxidase dose response was measured with Amplite™ Fluorimetric Xanthine Oxidase Assay Kit in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 14 µU/mL xanthine oxidase was detected with 30 minutes incubation time (n=3).