Amplite™ Fluorimetric Xanthine Oxidase Assay Kit

Red Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 11304 (200 assays)	Keep at -20°C 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. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. 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). The kit uses our Amplite™ Red substrate which makes it recordable in a dual mode, the fluorescent signal can be easily read by either a fluorescence microplate reader at Ex/Em = 530-570 nm/590 -600 nm (optimal Ex/Em = 540 nm/590 nm) or an absorbance microplate reader at 576±5 nm. With the Amplite™ Fluorimetric Xanthine Oxidase Assay Kit, we have detected as little as 0.15mU/mL xanthine oxidase in a 100 µL reaction volume.

Kit Key Features

Sensitive:	Detect as low as 0.15mU/mL XO in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

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	100µL (100 X)
Component E: Xanthine Oxidase Standard	1 vial (200 mU, lyophilized)
Component F: DMSO	1vial (200 µL)

Protocol for one 96-well plate

Brief Summary

XOstandards or test samples (50 µL)→ Add XO assay mixture (50 µL)→ Incubate at room temperature for 30-60 min → 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°C.

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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°C.

1.3 2U/mL Xanthine Oxidase (XO) stock solution: Add 100µL 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°C.

2. Prepare assay mixture:

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

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)	10µL
Xanthine (100X, Component D)	50µL
Assay Buffer (Component B)	5 mL
Total volume	5.08 mL

3. Prepare serially diluted XO standards (0 to 20mU/mL):

3.1 Add 10 µL of 2 U/mL XO stock solution (from Step 1.3) into 990 µL of Assay Buffer (Component B) to get 20 mU/mL XO standard solution.

3.2 Take 300 µL of 20 mU/mL XO standard solution to perform 1:2 serial dilutions to get 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156 and 0 mU/mL serially diluted XO standards.

3.3 Add XO standards and/or XO-containing test samples into a solid black 96-well microplate as described 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 1: Add the serially diluted xanthine oxidase standards from 0.156 mU/mL to 10 mU/mL into each well from XO1 to XO7 in duplicate.*
Note 2: High concentration of XO may cause reduced fluorescence signal due to the over oxidation of Amplite™ Red substrate (to a non-fluorescent product).

4. Run XO assay:

3.1 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.

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

3.3 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.
Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576±5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

4. Run 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 (XO standard curve).
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

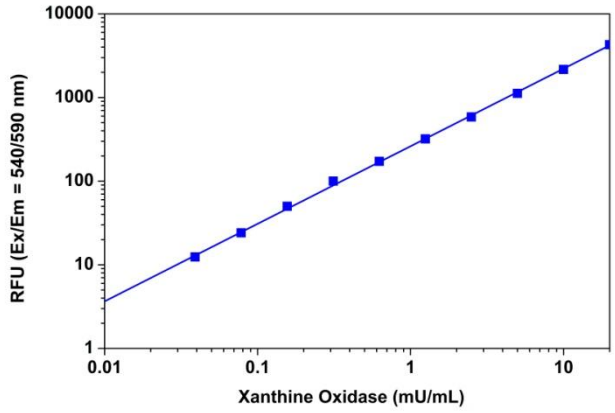

Figure 1. Xanthine oxidase dose response was measured with Amplite™ Fluorimetric Xanthine Oxidase Assay Kit in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.15mU/mL xanthine oxidase was detected with 30 minutes incubation time (n=3).

References

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