Amplite™ Fluorimetric Lysyl Oxidase Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 15255 (500 assays)	Keep in freezer Avoid exposure to light.	Fluorescence microplate readers

Introduction

Lysyl oxidase is an extracellular enzyme that catalyzes formation of aldehydes from lysine residues in collagen and elastin precursors. These aldehydes are highly reactive, and undergo spontaneous chemical reactions with other lysyl oxidase-derived aldehyde residues or with unmodified lysine residues. The chemical reactions result in cross-linking collagen and elastin, which is essential for stabilization of collagen fibrils and for the integrity and elasticity of mature elastin. The activity of Lysyl oxidase in biological samples is traditionally assessed by tritium release end-point assays using radio isotope labeled collagen or elastin substrates.

The Amplite™ Fluorimetric Lysyl Oxidase Assay Kit offers a sensitive fluorescent assay for detecting the activity of lysyl oxidase. It utilizes a proprietary LOX substrate that releases hydrogen peroxide detected using our Amplite™ ADHP substrate in HRP-coupled reactions. This method allows the detection of sub ng/mL lysyl oxidase and is much more sensitive than the currently available assays. It eliminates the interference that occurs in some biological samples and can be readily used to detect lysyl oxidase activity in cell extracts or solutions. Its signal can be easily read by either a fluorescence microplate reader at Ex/Em = 540/590 nm or an absorbance microplate reader at ~576 nm.

Kit Key Features

Sensitive:	Detect as low as 40 ng lysyl oxidase 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™ HRP Substrate (light sensitive)	1 vial
Component B: Assay Buffer	1 bottle (50 mL)
Component C: Horseradish Peroxidase	1 vial (50 units)
Component D: DMSO	1 vial (200 µL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare assay reaction mixture (50 µL) → Add lysyl oxidase standards or test samples (50 µL) → Incubate at 37 °C for 10-30 minutes → Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

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1. Prepare stock solutions:

Warning 1: *Amplite™ HRP Substrate is unstable in the presence of thiols such as DTT, glutathione (reduced form: GSH) and β-mercaptoethanol. The presence of thiols at concentration higher than 10 μM would significantly decrease the assay dynamic range.*

Warning 2: *Some detergents (such as Brij-35, Tween-20 and NP40), NADH and NADPH also interfere with the assay.*

1.1 250X Amplite™ HRP Substrate stock solution: Add 100 μL of DMSO (Component D) into the vial of Amplite™ HRP Substrate (Component A). The stock solution should be used promptly; any unused solution should be aliquoted and refrozen at -20 °C.
Note: Avoid repeated freeze-thaw cycles.

1.2 50 U/mL Horseradish Peroxidase stock solution: Add 1 mL 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.

2. Prepare assay reaction mixture:

Prepare assay reaction mixture according to the following tables and keep from light.

Table 1 2X Assay reaction mixture for one 96-well plate

Components	**Volume**
Amplite™ HRP Substrate stock solution (250X, from Step 1.1)	20 μL
50 U/mL Horseradish Peroxidase (from Step 1.2)	20 μL
Assay Buffer (Component B)	5 mL
Total volume	5 mL

Table 2 Layout of Lysyl Oxidase standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
LS1	LS1	….	….	….	….						
LS2	LS2										
LS3	LS3										
LS4	LS4										
LS5	LS5										
LS6	LS6										
LS7	LS7										

Note: LS= Lysyl Oxidase Standards, BL=Blank Control, TS=Test Samples.

Table 3. Reagent composition for each well

Lysyl Oxidase Standards	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 μL	Assay Buffer (Component B): 50 μL	50 μL

**Note1: Add the serially diluted Lysyl Oxidase standards from 0.04 ng to 4 μg into wells from LS1 to LS7 in duplicate.*

Note2: High concentration of Lysyl Oxidase may cause reduced fluorescence signal due to the over oxidation of Amplite™ HRP Substrate (to a non-fluorescent product).

3. Run lysyl oxidase assay in supernatants:

3.1 Add 50 μL of assay reaction mixture (from Step 2) into each well of lysyl oxidase standard, blank control, and test samples (see Step 2, Table 3) to make the total lysyl oxidase 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.

3.2 Incubate the reaction at 37 °C for 10 to 30 minutes, protected from light.

3.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 540/590 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 lysyl oxidase assay for cells:

The Amplite™ Fluorimetric Lysyl Oxidase Assay Kit can be used to measure the release of active lysyl oxidase from cells. The following is a suggested protocol that can be modified according to your specific research needs.

4.1 Prepare cells in a 96-well plate (50 - 100 µL/well), and activate the cells as desired. Harvest the cell media.
Note: The negative controls (media alone and non-activated cells) are included for measuring background fluorescence.

4.2 Add 50 µL of assay reaction mixture (from Step 2) into each well of the cell media (from Step 4.1), and those of lysyl oxydase standards (from Step 2).
Note: For a 384-well plate, add 25 µL of cell media and 25 µL of assay reaction mixture *into each well.*

4.3 Incubate the reaction at 37 °C for 10 to 30 minutes, protected from light.

4.4 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 530 to 570/590 to 600 nm (maximum Ex/Em = 540 /590 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 lysyl oxidase reactions. The typical data are shown in Figure 1. *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.*

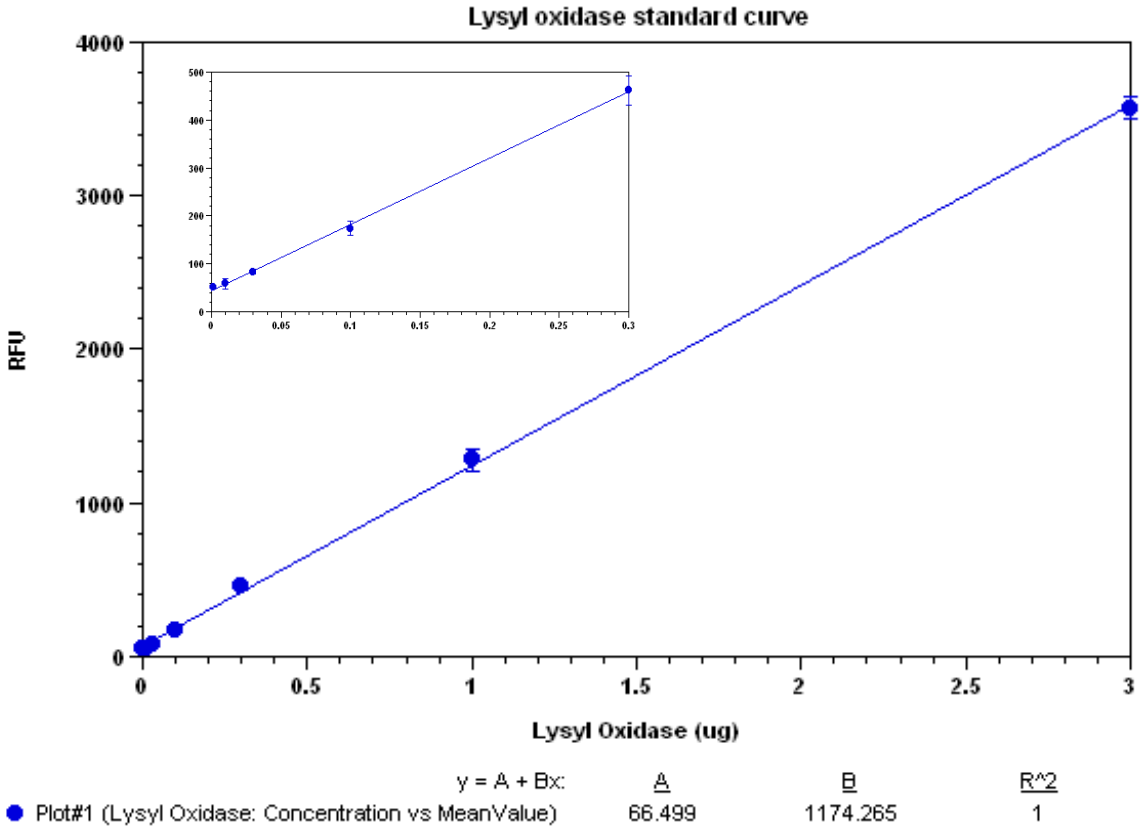

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Figure 1. Lysyl oxidase dose response was measured on a solid black 96-well with the Amplite™ Fluorimetric Lysyl Oxidase Assay Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 40 ng of lysyl oxidase can be detected with 30 minutes incubation (n=3). The insert shows the low levels of lysyl oxidase detection.

References

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