Amplite™ Fluorimetric Lysyl Oxidase Assay Kit

Red Fluorescence

Ordering Information:
Product Number: #15255 (500 assays)

Instrument Platform:
Fluorescence microplate readers

Storage Conditions:
Keep in freezer and avoid light

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. This results in cross-linking collagen and elastin that is essential for stabilization of collagen fibrils and for the integrity and elasticity of mature elastin. Lysyl oxidase has been identified as a possible tumor suppressor. Lysyl oxidase activity in biological samples is traditionally and most reliably assessed by tritium release end-point assays using radiolabeled collagen or elastin substrates involving laborious vacuum distillation of the released tritiated water. This kit offers a sensitive fluorescent assay for lysyl oxidase activity that utilizes 1,5-diaminopentane as substrate, and released hydrogen peroxide is detected using our Amplite™ HRP 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 fluorimetric assay for this enzyme activity. This method eliminates the interference that occurs in some biological samples and can be readily used to detect lysyl oxidase activity in cell culture experiments.

The Amplite™ Fluorimetric Lysyl Oxidase Assay Kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by either fluorescence microplate reader with Ex/Em = 530 to 570 nm/590 to 600 nm (maximum Ex/Em = 570 nm/590 nm) or absorbance microplate reader at 576±5 nm.

Kit Key Features

Sensitive:	The kit detect as low as 40 ng lysyl oxidase in solution.
Continuous:	Easily adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Component	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 1 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 min → Read fluorescence at Ex 570 nm/Em 590 nm

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

1. Prepare stock solutions:

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

1.2 50 U/ml 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 as single use aliquots and stored at -20°C.

2. Prepare assay reaction mixture:

2.1 Prepare Assay reaction mixture according to the following table and kept from light:

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

Components	**Volume**
Amplite™ HRP substrate stock solution (250X, from step 1.1)	20 uL
50 U/ml Peroxidase (from step 1.2)	200 uL
Assay Buffer (Component B)	4.7 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 Standard	**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 to4 ug into wells from LS1 to LS7 in duplicate.*

Note2: High concentration of Lysyl Oxidase may cause reduced fluorescence signal due to the overoxidation 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) to each well of the lysyl oxidase standard, blank control, and test samples (see step 2, table 3) so that the total lysyl oxidase assay volume is 100 µL/well
Note: For a 384-well plate, add 25 µL sample, 25 µL of assay reaction mixture *per well.*

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

3.3 Monitor the fluorescence increase with 530-570 nm (optimal at 570) excitation and 590-600 nm emission using a fluorescence plate reader.
Note: The contents of the plate can also be transferred to a white clear bottom plate and read by 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 for your specific research needs.

4.1 Prepare cells in 96-well plate (50-100 uL/well), and activate the cells as desired. Harvest the cell media.
Note1: 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) to 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 cell media, 25 µL of assay reaction mixture *per well.*

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

4.4 Monitor the fluorescence increase with 530-570 nm (optimal at 570) excitation and 590-600 nm emission using a fluorescence plate reader.

5. 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 lysyl oxidase reactions. The typical data are shown in Figure 1 (lysyl oxidase 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.

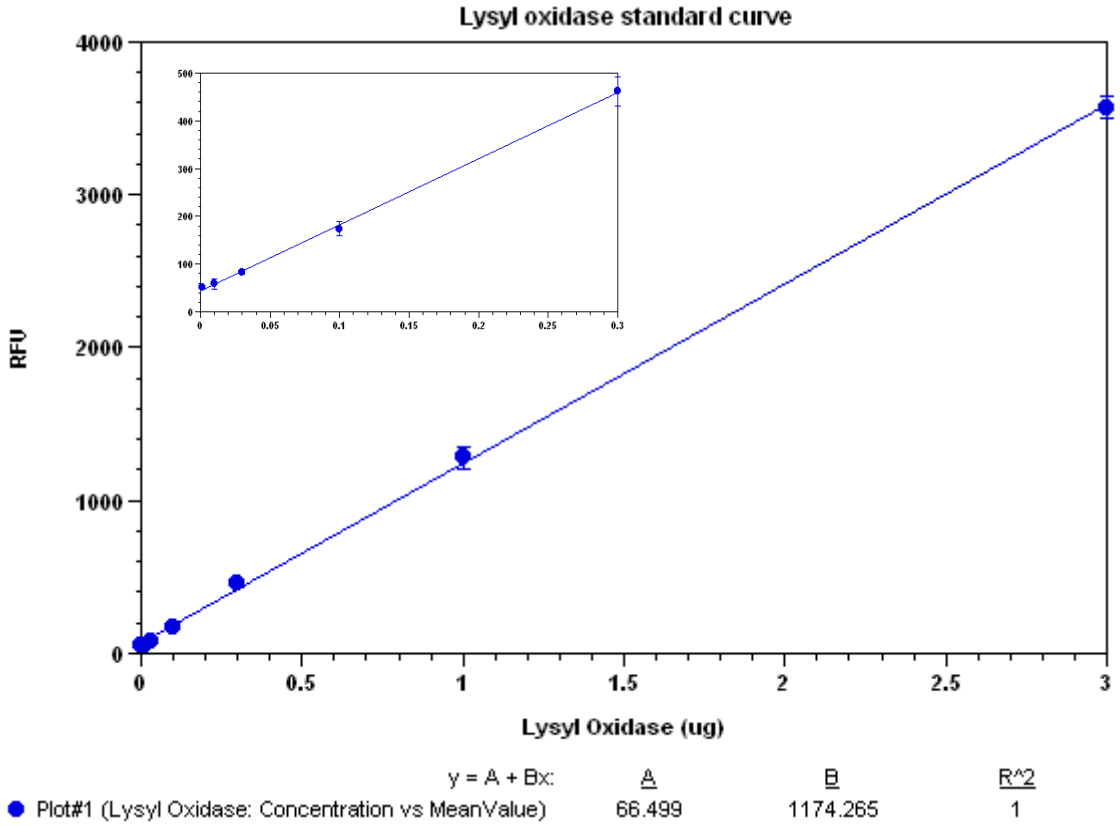

Figure 1. Lysyl oxidase dose response on 96-well black plate using a Gemini fluorescence microplate reader (Molecular Devices) measured with the Amplite™ Fluorimetric Lysyl Oxidase Assay Kit. As low as 40 ng of lysyl oxidase can be detected with 30 minutes incubation time (n=3). The insert shows the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

References:

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