Amplite™ Fluorimetric Lysyl Oxidase Assay Kit *Red Fluorescence*

Catalog number: 15255
Unit size: 500 Tests

Component	Storage	Amount
Component A: Amplite™ HRP Substrate (light sensitive)	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (50 mL)
Component C: Horseradish Peroxidase	Freeze (< -15 °C), Minimize light exposure	1 vial (50 units)
Component D: DMSO	Freeze (< -15 °C)	1 vial (200 µL)

OVERVIEW

Lysyl oxidase (LOX) 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 which 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 to measure LOX activity using our proprietary LOX substrate that releases hydrogen peroxide upon LOX oxidation. The amount of hydrogen peroxide released by the LOX oxidation is detected using our Amplite™ HRP substrate in the 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. Please note that the kit does not include the lysyl oxidase enzyme.

AT A GLANCE

Protocol Summary

1. Prepare lysyl oxidase standards or test samples (50 µL)
2. Add lysyl oxidase working solution (50 µL)
3. Incubate at 37°C for 10 - 30 minutes
4. Monitor fluorescence intensity at Ex/Em = 540/590 nm

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

KEY PARAMETERS

Fluorescence microplate reader

Excitation	540 nm
Emission	590 nm
Cutoff	570 nm
Recommended plate	Solid black

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

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™ HRP Substrate stock solution (250X)

Add 100 µL of DMSO (Component D) into the vial of Amplite™ HRP Substrate (Component A).

Note 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 will significantly decrease the assay dynamic range. Some detergents (such as Brij-35, Tween-20 and NP40), NADH, and NADPH can also interfere with this assay.

2. Horseradish Peroxidase stock solution (50 U/mL)

Add 1 mL of Assay Buffer (Component B) into the vial of Horseradish Peroxidase (Component C).

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/15255

Lysyl Oxidase standard

Prepare lysyl oxidase standards by serial dilution to obtain standards from 0.04 to 4 µg/mL (LS1 - LS7).Note: Lysyl oxidase standard is not provided in this kit. It can be purchased from R&D Sytems (2639-AO-010 or 6069-AO-010).

PREPARATION OF WORKING SOLUTION

Amplite™ HRP Substrate working solution

Add 20 µL of Amplite™ HRP Substrate stock solution (250X) and 20 µL of Horseradish Peroxidase (50 U/mL) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.04 mL.

Note The working solution is not stable, use it promptly and avoid direct exposure to light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of lysyl oxidase standards and test samples in a solid black 96-well microplate. LS = lysyl oxidase standard (LS1-LS7, 0.04 to 4 µg/mL); BL = blank control; TS = test sample.

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

Table 2. Reagent composition for each well. Note that high concentration of Lysyl Oxidase may cause reduced fluorescence signal due to the over oxidation of Amplite™ HRP Substrate (to a non-fluorescent product). Lysyl Oxidase standards are for positive control only, and should not be relied on as a quantitation standard for enzyme activity.

Well	Volume	Reagent
LS1-LS7	50 µL	Serial Dilution (0.04 to 4 µg/mL)
BL	50 µL	Assay Buffer (Component B)

TS	50 µL	Test Sample

Lysyl Oxidase assay in supernatants

1. Prepare lysyl oxidase standards (LS), blank controls (BL) and test samples (TS) according to the layout provided in Table 1 and Table 2. For a 384-well plate, use 25 µL of each corresponding reagent instead of 50 µL.

2. Add 50 µL of lysyl oxidase working solution into each well of lysyl oxidase standard, blank control, and test samples to make the total lysyl oxidase assay volume of 100 µL/well. For a 384-well plate, add 25 µL of working solution into each well for a total volume of 50 µL.

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

4. 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. Note though that the absorption detection has lower sensitivity compared to fluorescence reading.

Lysyl Oxidase assay for cells

1. Prepare cells in a 96-well plate (50 - 100 µL/well), and activate the cells as desired. For a 384-well plate, use 25 µL/well instead. Harvest the cell media.

   Note The negative controls (media alone and non-activated cells) are included for measuring background fluorescence.

2. Add 50 µL of lysyl oxidase working solution into each well of the cell media (from previous step) and well of lysyl oxidase standards (see Table 1). For a 384-well plate, add 25 µL of working solution into each well instead.

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

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, cut off 570 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

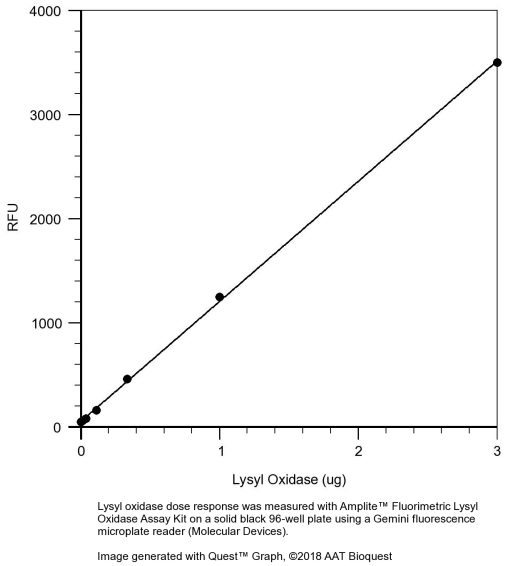

Figure 1. Lysyl oxidase dose response was measured with Amplite™ Fluorimetric Lysyl Oxidase Assay Kit on a solid black 96-well plate using a Gemini fluorescence microplate reader (Molecular Devices).

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