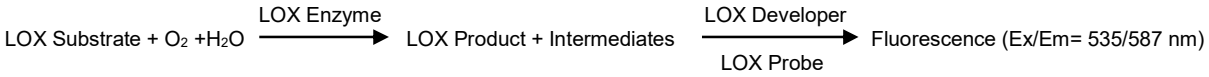
Lysyl Oxidase Activity Assay Kit (Fluorometric) (#BN01101)

(Catalog # BN01101; 100 assays; Store at -20°C)

I. Introduction:

Lysyl oxidase is a cell-secreted amine oxidase that catalyzes the final step of collagen and elastin cross-linking. As a result, lysyl oxidase is required for the biosynthesis of functional extracellular matrices. Inhibition of lysyl oxidase can result in osteolathyrism, a condition characterized by fragile connective tissue and paralysis. Recent work has also suggested lysyl oxidase to play an important role in cancer development: Lysyl oxidase promotes tumor growth and progression *in vivo*, cancer cell invasion, and premetastatic niche formation. Assay Genie's Lysyl Oxidase Activity Fluorometric Assay Kit allows for quantitative evaluation of lysyl oxidase activity of purified enzyme and its inhibitors as well as secreted endogenous enzyme in cell media. Enzyme activity is detected upon oxidation of substrate, which results in release of reaction intermediates. Reaction intermediates are subsequently detected by Assay Genie's LOX Probe, resulting in fluorescent signal that can be measured at Ex/Em= 535/587 nm. Each kit includes a positive control as well as a specific lysyl oxidase inhibitor that allows the user to correct each sample for any non-specific signal. Lysyl Oxidase Activity Fluorometric Assay Kit can detect as little as 40 ng of recombinant enzyme *in vitro*.



II. Applications:

- Measurement of Lysyl Oxidase activity of purified proteins
- Quantitative analysis of Lysyl Oxidase mutants and inhibitors
- Quantitative evaluation of Lysyl Oxidase activity in cell media

III. Sample Type:

- Purified protein, cell media.

IV. Kit Contents:

Components	BN01101	Cap Code	Part Number
LOX Assay Buffer	45 ml	NM	BN01101-1
LOX Substrate	100 µl	Clear	BN01101-2
LOX Developer	1 vial	Green	BN01101-3
LOX Probe (in DMSO)	100 µl	Red	BN01101-4
LOX Positive Control	1 vial	Blue	BN01101-5
LOX Inhibitor	1 vial	Purple	BN01101-6
LOX Standard (0.88 M)	100 µl	Yellow	BN01101-7

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom, low-medium binding
- Spectrophotometer
- Purified wild type or mutant Lysyl Oxidase protein, cell media samples.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **LOX Assay Buffer:** Warm to 37 ºC temperature before use. Store at RT.
- **LOX Substrate:** Ready to use. Store at -20°C.
- **LOX Developer:** Reconstitute with 1 ml of LOX Assay Buffer. Store at -20°C.
- **LOX Positive Control:** Reconstitute with 33 µl of H_2O. *Aliquot and immediately store at -80°C*. Avoid multiple freeze-thaw cycles.
- **LOX Inhibitor:** Reconstitute with 220 µl of LOX Assay Buffer. Store at -20°C.
- **LOX Standard (0.88M):** Store at -20°C. Before each assay, dilute 4.3 µl 0.88M LOX Standard in 495.7 µl LOX Assay Buffer to make 7.5 mM LOX Standard. Further dilute 10 µl of 7.5 mM LOX Standard in 990 µl LOX Assay Buffer to make 75 µM LOX Standard working stock to use as assay spike standard. *Do not store LOX Standard dilutions; make fresh LOX Standard working stock each time*.
- **LOX Probe (in DMSO):** Make sure the probe is completely thawed at RT prior to use. Store at -20°C.

VII. Lysyl Oxidase Activity Assay Protocol:

1. Sample Preparation:

Prepare the following reactions in a black microplate:

a. Positive Control: Add 3 µl of LOX positive control to 47 µl LOX Assay Buffer.

b. Sample: Dilute sample to desired concentration using LOX Assay Buffer and adjust the volume to 50 µl. If using cell media as sample, add no more than 10 µl per reaction and adjust the volume to 50 µl with LOX Assay Buffer.

c. Sample + Spike Standard: Mix sample with 2 µl 75 µM LOX Standard; adjust the volume to 50 µl with LOX Assay Buffer.

d. Negative Control: Mix sample with 2 µl LOX inhibitor; adjust the volume to 50 µl with LOX Assay Buffer.

e. Reagent Control: Add 50 µl LOX Assay Buffer only to control for assay reagent stability.

Important! Conditioned cell media exhibits a matrix effect. Therefore, when running cell media sample for quantitative evaluation, it is always necessary to run the same volume of cell media in sample, spike standard, and negative control wells side by side.

Notes:

a. Do not store enzyme/inhibitor/sample dilutions; discard the dilutions instead.

b. For cell culture application, use serum-free phenol red-free media during cell treatment because serum and phenol red interfere with LOX probe.

c. For unknown enzymes and media, we suggest testing several doses to ensure the reading is within the linear range of the assay.

d. The release of Lysyl Oxidase into cell media will vary depending on cell type and treatment. If the activity is low, you may concentrate the cell media up to 20X using 10 kDa cut off spin columns. *Aim to use no more than 10 µl cell media per assay*.

2. Reaction Mix: Mix enough reagents for the number of assays to be performed:

	Sample Reaction Mix	
	(1 assay)	(10 assays)
LOX Substrate	1 µl	10 µl
LOX Developer	2 µl	20 µl
LOX Probe	0.5 µl	5 µl
LOX Assay Buffer	46.5 µl	465 µl

Mix and add 50 µl of the Sample Reaction Mix to each well containing the Positive Control, Test Samples, Spike Standard, Negative Control, and Reagent Control.

3. Measurement: Measure fluorescence (Ex/Em = 535/587 nm) in kinetic mode every 30 seconds for at least 60 minutes at 37 °C in a black plate.

4. Calculations: For test sample, calculate the corrected sample fluorescence at each time point by subtracting Inhibitor Negative Control RFU from sample and spike standard reading: F_S = RFU_S - RFU_{Inh} and $F_{S+Spike}$ = $RFU_{S+Spike}$ – RFU_{Inh}. Calculate pmol of LOX Product generated at each time point using Equation 1 below. Plot pmol LOX Product on the y-axis vs. time (in minutes) on the x-axis and determine the slope (pmol/min) of the linear portion of the reaction curve. Calculate sample enzyme acitivity using Equation 2 below.

Equation 1: $\textbf{Sample pmol product}\ \text{generated at time}\ (\boldsymbol{t}) = \left(\frac{Fs}{(Fs+spike)-Fs}\right) \times \mathbf{150}$

Equation 2: $\textbf{Sample Enzyme Activity} = \left(\frac{slope}{V}\right) \times \boldsymbol{D}\ (\boldsymbol{pmol/min/ml} \equiv \boldsymbol{\mu U/ml})$

Where: **V** = Sample volume added into the reaction well (ml)
D = Dilution Factor
Slope = pmol/min (from the linear range of the activity curve)
150 = added spike standard

Unit Definition: One unit of Lysyl Oxidase is the amount of enzyme that consumes 1.0 µmol of LOX Substrate per min at 37°C.

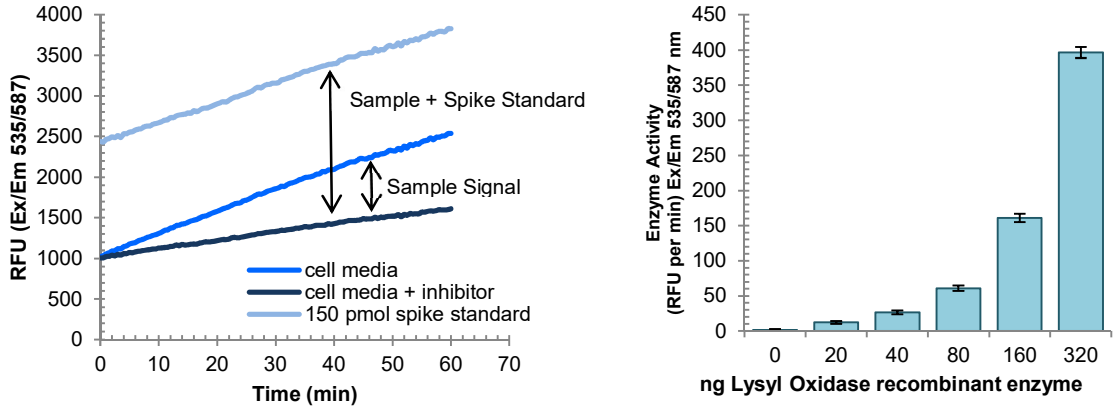


Figure: a) Enzyme activity at varying concentrations of recombinant Lysyl Oxidase b) representative activity curve for cell media sample and cell media spike standard at 37°C, where cell media is test sample, cell media + inhibitor is negative control and 150 pmol spike standard is sample + spike standard.

FOR RESEARCH USE ONLY! Not to be used on humans.