ab284573– Lysyl Oxidase Activity Assay Kit (Fluorometric)

For the measurement of Lysyl Oxidase activity of purified enzyme in addition to secreted endogenous enzyme in cell media.
For research use only - not intended for diagnostic use.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
Lysyl Oxidase Assay Buffer	45 mL	-20°C
Developer Solution V	1 vial	-20°C
Lysyl Oxidase Inhibitor	1 vial	-20°C
Lysyl Oxidase Positive Control	1 vial	-20°C
OxiRed™ Probe	0.2 ml	-20°C
H2O2 Standard	100 µl	-20°C
Lysyl Oxidase Substrate	100 µl	-20°C

PLEASE NOTE: Lysyl Oxidase Positive Control was previously labelled as LOX Positive Control, and OxiRed™ Probe as LOX Probe (in DMSO), and H2O2 Standard as LOX Standard (0.88 M), and Lysyl Oxidase Substrate as LOX Substrate, and Lysyl Oxidase Assay Buffer as LOX Assay Buffer, and Developer Solution V as LOX Developer, and Lysyl Oxidase Inhibitor as LOX Inhibitor. The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Lysyl Oxidase Assay Buffer: Warm to 37 °C temperature before use. Store at -20°C.

Lysyl Oxidase Substrate: Ready to use. Store at -20°C

Developer Solution V: Reconstitute with 1 ml of Lysyl Oxidase Assay Buffer. Store at -20°C.

Lysyl Oxidase Positive Control: Reconstitute with 33 µl of H_2O. Aliquot and immediately store at -20°C. Avoid multiple freeze-thaw cycles

Lysyl Oxidase Inhibitor: Reconstitute with 220 µl of Lysyl Oxidase Assay Buffer. Store at -20°C.

H2O2 Standard: Store at -20°C. Before each assay, dilute 4.3 µl H2O2 Standard in 495.7 µl Lysyl Oxidase Assay Buffer to make 7.5 mM H2O2 Standard. Further dilute 10 µl of 7.5 mM H2O2 Standard in 990 µl Lysyl Oxidase Assay Buffer to make 75 µM H2O2 Standard working stock to use as assay spike standard.

***Δ Note**: Do not store H2O2 Standard dilutions; make fresh H2O2 Standard working stock each time.*

OxiRed™ Probe: Make sure the OxiRed™ Probe is completely thawed at RT prior to use. Store at -20°C.

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Assay Protocol

Sample preparation:

Prepare the following reactions in a black microplate:

1. Positive Control: Add 3 µl of Lysyl Oxidase Positive Control to 47 µl Lysyl Oxidase Assay Buffer.
2. Sample: Dilute sample to desired concentration using Lysyl Oxidase 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 Lysyl Oxidase Assay Buffer.
3. Sample + Spike Standard: Mix sample with 2 µl 75 µM H2O2 Standard; adjust the volume to 50 µl with Lysyl Oxidase Assay Buffer.
4. Negative Control: Mix sample with 2 µl Lysyl Oxidase Inhibitor; adjust the volume to 50 µl with Lysyl Oxidase Assay Buffer.
5. Reagent Control: Add 50 µl Lysyl Oxidase Assay Buffer only to control for assay reagent stability.

***Δ Note**: 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.*

Δ Note: Do not store enzyme/inhibitor/sample dilutions; discard the dilutions instead.

For cell culture application, use serum-free phenol red-free media during cell treatment because serum and phenol red interfere with OxiRed™ Probe.

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

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 (BV cat# 1997). Aim to use no more than 10 µl cell media per assay.

Reaction Mix:

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

	Reaction Mix – 1 assay	Reaction Mix – 10 assays
Lysyl Oxidase Substrate	1 µl	10 µl
Developer Solution V	2 µl	20 µl
OxiRed™ Probe	0.5 µl	5 µl
Lysyl Oxidase Assay Buffer	46.5 µl	465 µl

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

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.

Calculation:

1. For test sample, calculate the corrected sample fluorescence at each time point by subtracting Inhibitor Negative Control RFU from sample and spike standard reading: FS = RFUS - RFUInh and FS+Spike = RFUS+Spike – RFUInh.
2. Calculate pmol of LOX Product generated at each time point using Equation 1 below.
3. 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.
4. Calculate sample enzyme acitivity using Equation 2 below.

$$\text{Sample pmol product generated at time (t)} = \frac{Fs}{(Fs+spike)-\mathrm{Fs}} \times 150$$

$$\text{Sample enzyme activity} = \left(\frac{slope}{V}\right) \times D \; (pmol/min/ml = \mu U/ml)$$

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

Technical Support

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