



TECHNICAL MANUAL

Lysyl Oxidase Activity Assay Kit (Fluorometric)

- **SKU CODE:** ARMA00229
- **SIZE:** 100 Wells
- **DETECTION PRINCIPLE:** Fluorometric
- **RUO:** Research-Use-Only

Lysyl Oxidase Activity Assay Kit (Fluorometric)

Please read entire manual carefully before starting experiment.

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1. Key Features

- Measures lysyl oxidase (LOX) activity with a sensitivity below 5 μ Units per well.
- Fluorometric detection (Ex/Em = 535/587 nm), measured kinetically.
- Internal standard protocol to account for sample matrix effects – no separate standard curve required.
- Includes Active LOX positive control and LOX Inhibitor for background control.
- Suitable for cell extracts and cell culture media.
- 100 wells.

2. Storage & Expiry

Store unopened kit at -20°C. Bring all components to room temperature before using. Centrifuge all vials for a few seconds before opening.

- **Assay Buffer:** Ready to use as supplied. Store at 4°C.
- **Diaminopentane (LOX Substrate):** Ready to use as supplied. Comes as a 1 M solution. Store at -20°C.
- **Peroxidase:** Reconstitute with 1 ml of Assay Buffer. Divide into convenient portions and store at -20°C to avoid multiple freeze/thaw cycles.
- **ADHP Solution:** Comes as a 10 mM solution in DMSO. Make sure DMSO is completely melted before pipetting. Store at -20°C.
- **Active LOX:** Reconstitute with 33 μ l of DI water. Divide into convenient portions and store at -20°C to -80°C to avoid multiple freeze/thaw cycles.
- **LOX Inhibitor:** Dissolve with 220 μ l of DI water. Store at -20°C.
- **H₂O₂:** Comes as an 8.8 mM solution. Before an assay, transfer 3 μ l of 8.8 mM H₂O₂ to 349 μ l DI water giving a 75 μ M H₂O₂ solution. Keep on ice temporarily. Discard any unused diluted H₂O₂. It will decompose relatively rapidly so should not be stored and reused.

3. Product Description

Lysyl oxidase (LOX) is an enzyme that has a role in the formation and maintenance of the extracellular matrix with significant implications for metabolic health. A copper-dependent amine oxidase, LOX is primarily secreted by smooth muscle cells and fibroblasts, catalyzing the crosslinking of collagen and elastin fibers. Initially recognized for its structural functions, recent research has unveiled correlations between LOX activity and various physiological and pathological processes, including adipose tissue function, glucose metabolism and type 2 diabetes, fibrosis in several tissues and cardiovascular health. Inhibition of LOX has shown promise in animal models for reducing metastasis and improving metabolic outcomes but, given the essential nature of LOX in normal tissue development and function, consideration must be given to potential side effects of LOX modulation. Assay Genie's Lysyl Oxidase Activity Assay Kit is a simple, sensitive means of measuring LOX activity with a sensitivity below 5 μ Units per well.

Assay Principle

In the presence of lysyl oxidase activity, hydrogen peroxide is formed during substrate oxidation. The hydrogen peroxide produced is utilized by peroxidase to convert ADHP, an almost colorless, non-fluorescent compound, into resorufin with a dramatic increase in color and fluorescence.

4. Applications

Measurement of lysyl oxidase activity in cell extracts or cell culture media into which lysyl oxidase has been released by cells during the culture period.

5. Kit Contents

Component	Size	Cap Colour
Assay Buffer	45 ml	NM
Diaminopentane	100 µl	Clear
Peroxidase	Lyophilized	Green
ADHP Solution	100 µl	Red
Active LOX	Lyophilized	Blue
LOX Inhibitor	Lyophilized	Purple
H ₂ O ₂	100 µl	Yellow

6. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

7. Assay Procedure

1. **Turn on a plate reader.** Set it to 37°C. Configure it to measure fluorescence using 535 nm excitation and 587 nm emission every 30-60 seconds for 60 minutes. Warm Assay Buffer to 37°C.
2. **Sample Considerations:** Samples are typically cell extracts or cell culture media into which lysyl oxidase has been released by cells during the culture period. Most media exhibit a strong matrix effect. Do not use media containing serum or phenol red as each interferes with the assay.
3. **Assay Standard:** Rather than use a separate standard curve, due to the matrix effect of the samples, the assay utilizes an internal standard protocol where each sample is run in triplicate, with one of each triplicate having 150 picomoles of H₂O₂ included as an internal standard and another including a potent enzyme inhibitor. Remove the diluted H₂O₂ prepared above from ice and allow it to warm to room temperature.
4. **Positive Control:** Add 3 µl of Active LOX to a well and adjust the volume to 50 µl with Assay Buffer.
5. **Samples:** Apply up to 48 µl of each sample (up to 10 µl cell culture media, maximum) to wells in a (preferably) black 96-well plate in triplicate and adjust the volume to 48 µl with Assay Buffer.
6. **Internal Standard:** Transfer 2 µl of (diluted) 75 µM H₂O₂ to one of each sample triad.
7. **Background Control:** Transfer 2 µl LOX Inhibitor to one of each sample triad.

Notes:

- At this point each sample should be present in 3 wells: sample only, sample + internal standard and sample + LOX Inhibitor.
 - The amount of lysyl oxidase in cell media will vary widely depending on cell type and treatment regime. Samples with low activity can be concentrated using a 10 kDa MWCO centrifugal filter.
8. **Initiate Reaction:** Each well requires 50 µl of Reaction Mix. Prepare sufficient material for the total number of wells to be analyzed, each well containing:

Reaction Mix	Volume per well
Assay Buffer	46.5 μ l
Diaminopentane	1.0 μ l
Peroxidase	2.0 μ l
ADHP Solution	0.5 μ l

Mix and add 50 μ l of Reaction Mix to each well containing sample, sample + internal standard, sample + LOX Inhibitor and positive control.

9. **Measurement:** Place the plate in the plate reader and begin measuring fluorescence as set up in step 1 above.

8. Calculation

For each well, identify a region which is linear and determine the slope of the linear region. The sample and sample + internal standard should be parallel to each other. It may take a few minutes for the difference between them to stabilize. At any time point after the difference is constant, determine the difference. This is the instrument response to 150 picomoles of peroxide. Subtract the slope of the background control for each sample from the slope of the sample alone to obtain a background-corrected enzyme activity slope. Divide the difference in RFU between the sample alone and the sample + internal standard by 150 to determine the RFU/picomole peroxide. Divide the slope of the background-corrected sample alone by the RFU/picomole just calculated to give the enzyme rate in picomoles/minute (μ Units) for each sample well.

To convert to enzyme activity in the original samples:

- A. Divide the enzyme activity in a well by the amount of sample applied to the well (in μ l)
= μ Units LOX activity/ μ l sample applied to the well.
- B. If a sample was diluted due to very high enzyme activity, multiply the μ Units LOX activity/ μ l sample applied to the well by the dilution factor.

C. For cell culture samples, multiply by the total cell media volume to get total μ Units of enzyme activity.

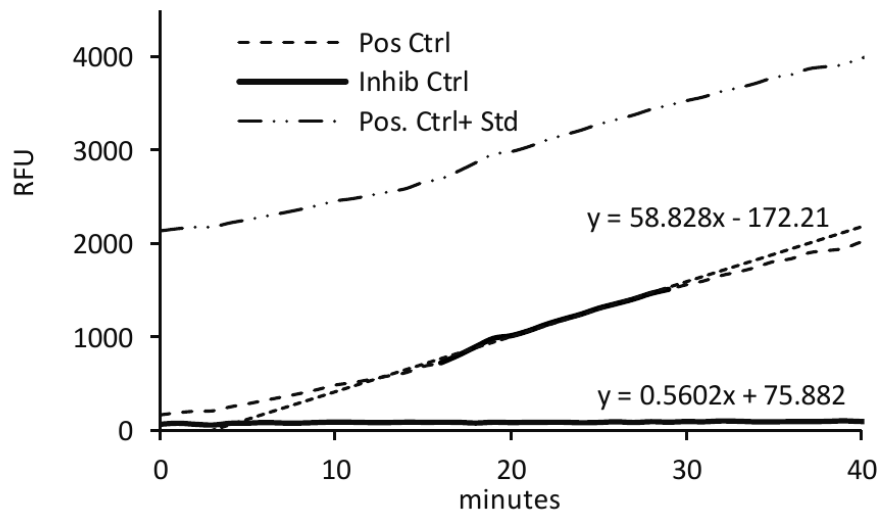
D. Divide by the # of cells per dish to obtain Units of LOX activity/cell or cell multiple.

9. Materials Required (But Not Provided)

- Fluorescence microplate reader capable of kinetic measurement at Ex/Em = 535/587 nm, set to 37°C
- 96-well plate (black plates preferred)
- Deionized (DI) water
- Pipettes and pipette tips
- Ice
- 10 kDa MWCO centrifugal filter (optional, for concentrating low-activity samples)

10. Example

Typical Results



Kinetic fluorescence (RFU) readings over time for the positive control (Pos Ctrl), inhibitor control (Inhib Ctrl) and positive control + internal standard (Pos. Ctrl + Std).

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

