Technical Bulletin

Lysyl Oxidase (LOX) Assay Kit

Catalogue number MAK555

Product Description

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 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.

The Lysyl Oxidase Assay Kit offers a sensitive fluorescent assay to measure LOX activity using a LOX substrate that releases hydrogen peroxide upon oxidation. The amount of hydrogen peroxide released by the LOX oxidation is detected using HRP substrate in the HRP-coupled reactions. This allows the detection of sub ng/mL lysyl oxidase. This method eliminates the interference that occurs in some biological samples and can be readily used to detect lysyl oxidase activity in cell culture samples. Please note that the kit does not include the lysyl oxidase enzyme.

Components

The kit is sufficient for 500 colorimetric assays in 96-well plates.

- HRP Substrate — 1 Vial
  Catalogue Number MAK555A
- Assay Buffer — 50 mL
  Catalogue Number MAK555B
- Horseradish Peroxidase — 1 Vial
  Catalogue Number MAK555C
- DMSO — 200 µL
  Catalogue Number MAK555D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories.
- Fluorescence multiwell plate reader.
- Black, flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Lysyl Oxidase Standard
  (R&D Systems 2639-AO-010)
- PBS with 0.1% BSA

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Preparation of Stock Solutions

HRP Substrate Stock Solution (250X): Add 100 µL of DMSO into the vial of HRP Substrate.

Note: 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.

Horseradish Peroxidase Stock Solution (50 U/mL): Add 1 mL of Assay Buffer into the vial of Horseradish Peroxidase.

Preparation of Standard Solution

Note: Lysyl oxidase standard is not provided in this kit. It can be purchased from R&D Systems (2639-AO-010).

Prepare Lysyl oxidase standards by serial dilution to obtain standards from 0.04 to 4 µg/mL (LS1 - LS7) as per Table 1. Use PBS with 0.1% BSA as the dilution buffer.

Table 1.

Dilution of Lysyl Oxidase Standards

Dilution	LOX Standard Volume (µL)	Serial Dilution Source	PBS Volume (µL)	Conc (µg/mL)
LS1	4	from 300 µg/mL stock	296	4
LS2	150	From LS1	150	2
LS3	150	From LS2	150	1
LS4	150	From LS3	150	0.5
LS5	150	From LS4	150	0.25
LS6	150	From LS5	150	0.125
LS7	150	From LS6	150	0.062

Preparation of HRP Substrate Working Solution

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

Note: The working solution is unstable, use it promptly and protect from light.

Assay Reaction for Supernatants

1. Prepare lysyl oxidase standards, blank controls and test samples by adding 50 µL of each into separate wells of a 96-well plate. For a 384-well plate, use 25 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.
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 $\lambda_{Ex}/\lambda_{Em}$ = 540/590 nm.

Assay Reaction 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) should be 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. 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 $\lambda_{Ex}/\lambda_{Em}$ = 530 to 570/590 to 600 nm (maxi $\lambda_{Ex}/\lambda_{Em}$ = 540/590 nm, cut off 570 nm).

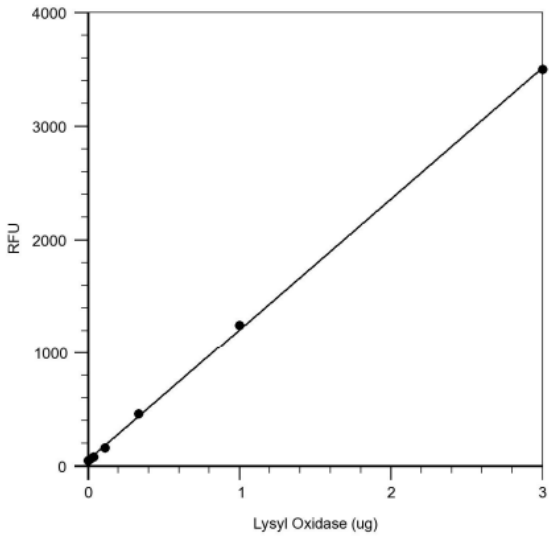
Results

1. The reading (RFU) obtained from the blank well is used as a negative control.
2. Subtract the blank value from the standards reading to obtain the baseline corrected values.
3. Calculate the mean value of the duplicate readings for each standard and sample. Using the fluorescence intensity, determine the change between the control and sample.
4. If the protein in the sample was quantified, the LOX activity levels can be compared relatively to the amount of protein present in the sample and incubation time (µg/mL/min).

Note: LOX standards in this assay are used for positive control only and should not be relied on as a quantitation standard for enzyme activity.

Figure 1

Lysyl oxidase dose response was measured with the Lysyl oxidase assay kit on a black 96-well plate.



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