Lysyl Oxidase Fluorimetric Assay Kit

Catalog No. KM0100

Detection and Quantification of Lysyl Oxidase in Biological Samples.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.
INTRODUCTION

Lysyl oxidase is an extracellular copper enzyme that catalyzes the conversion of lysine residues in collagen and elastin into aldehydes, which are highly reactive. These aldehydes spontaneously react with other aldehyde residues derived from lysyl oxidase or unmodified lysine residues, resulting in cross-linking of collagen and elastin. This is important for elasticity and integrity of elastin and stabilization of collagen. Upregulation of lysyl oxidase in tumor cells promotes metastasis of tumors while inhibition can lead to lathyrisim.

This assay detects activity of lysyl oxidase and is more sensitive than currently available assays. It can readily detect lysyl oxidase activity in cell extracts or solutions and eliminates interference from biological samples. In the assay, lysyl oxidase reacts with proprietary LOX substrate to form hydrogen peroxide. A HRP-coupled reaction then converts the hydrogen peroxide and ADHP substrate into a red fluorescent product, resorufin. Signals increase with increasing lysyl oxidase activity, and can be read with fluorescence microplate reader at excitation at 520 and emission at 590 nm or an absorbance microplate reader at 576 nm.

FEATURES

**Number of Assays:** 200 Assays

**Fluorimetric Dynamic Range:** Detect as low as 40 ng lysyl oxidase in solution

Ex/Em = 540/590 nm

STORAGE & HANDLING

Upon receipt, store all components in the original box at -20°C, protected from light. The kit should be stable for at least six months.

**Note:** Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
KIT CONTENTS & STOCK PREPARATION

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Well Microplate</td>
<td>2 plates</td>
<td>RT</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>1 vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>1 bottle (50 mL)</td>
<td>-20°C</td>
</tr>
<tr>
<td>HRP</td>
<td>1 vial (50 U)</td>
<td>-20°C</td>
</tr>
<tr>
<td>DMSO</td>
<td>1 vial (200 uL)</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

**Note:** If not running 200 assays at once, aliquot and store reagents under conditions according to this table. Use within 2 months.

MATERIALS NOT PROVIDED

Pipetting devices, tubes, and microplate reader.

ASSAY RESTRICTIONS

- This kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in performance of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.
REAGENT & SAMPLE PREPARATIONS FOR 1 PLATE

Please read the manual in its entirety before proceeding with the assay.

**Note:** Warm all reagents to room temperature before use and protect from light. Aliquot and store the reagents at -20°C if not all of it is used.

1. **Prepare stock solutions**

**Warning 1:** Dye Reagent 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 would significantly decrease the assay dynamic range.

**Warning 2:** Some detergents (such as Brij-35, Tween-20 and NP40), NADH and NADPH also interfere with the assay.

1.1 250X Dye Reagent stock solution: Add 100uL of DMSO into the vial of Dye Reagent. The stock solution should be used promptly; any unused solution should be aliquoted and refrozen at -20°C.

**Note:** Avoid repeated freeze-thaw cycles.

1.2 50 U/mL Horseradish Peroxidase stock solution: Add 1 mL of Assay Buffer into the vial of HRP.

**Note:** The unused HRP solution should be divided into single use aliquots and stored at -20°C.

2. **Prepare assay reaction mixture**

Prepare assay reaction mixture according to the following tables and keep from light.

**Table 1:** 2X Assay Reaction Mixture for one 96-well plate

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye Reagent (250X, from Step 1.1)</td>
<td>20 μL</td>
</tr>
<tr>
<td>50 U/mL Horseradish Peroxidase (from Step 1.2)</td>
<td>20 μL</td>
</tr>
<tr>
<td>Assay Buffer (Component B)</td>
<td>5 mL</td>
</tr>
<tr>
<td>Total volume</td>
<td>5 mL</td>
</tr>
</tbody>
</table>
**Table 2:** Layout of Lysyl Oxidase Standards and test samples in a solid black 96-well microplate

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BL</td>
<td>BL</td>
<td>TS</td>
<td>TS</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>LS1</td>
<td>LS1</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>LS2</td>
<td>LS2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>LS3</td>
<td>LS3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>LS4</td>
<td>LS4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>LS5</td>
<td>LS5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>LS6</td>
<td>LS6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>LS7</td>
<td>LS7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: LS= Lysyl Oxidase Standards, BL=Blank Control, TS=Test Samples.*

**Table 3:** Reagent composition for each well

<table>
<thead>
<tr>
<th>Lysyl Oxidase Standards</th>
<th>Blank Control</th>
<th>Test Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Dilutions*: 50 μL</td>
<td>Assay Buffer: 50 μL</td>
<td>50 μL</td>
</tr>
</tbody>
</table>

*Note 1: Add the serially diluted Lysyl Oxidase standards from 0.04 ng to 4 μg into wells from LS1 to LS7 in duplicate.*

*Note 2: High concentration of Lysyl Oxidase may cause reduced fluorescence signal due to the over oxidation of Dye Reagent (to a non-fluorescent product).*
ASSAY PROTOCOL FOR 1 PLATE

3. Run Lysyl oxidase assay in supernatants

3.1 Add 50 μL of assay reaction mixture (from Step 2) into each well of lysyl oxidase standard, blank control, and test samples (see Step 2, Table 3) to make the total lysyl oxidase assay volume of 100 μL/well.  
Note: For a 384-well plate, add 25 μL of sample and 25 μL of assay reaction mixture into each well.

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

3.3 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. The absorption detection has lower sensitivity compared to fluorescence reading.

4. Run Lysyl oxidase assay for cells

The Lysyl Oxidase Assay Kit can be used to measure the release of active lysyl oxidase from cells. The following is a suggested protocol that can be modified according to your specific research needs.

4.1 Prepare cells in a 96-well plate (50 - 100 μL/well), and activate the cells as desired. Harvest the cell media.
Note: The negative controls (media alone and non-activated cells) are included for measuring background fluorescence.

4.2 Add 50 μL of assay reaction mixture (from Step 2) into each well of the cell media (from Step 4.1), and those of lysyl oxidase standards (from Step 2).
Note: For a 384-well plate, add 25 μL of cell media and 25 μL of assay reaction mixture into each well.

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

4.4 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)
STANDARD CURVE

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with lysyl oxidase reactions. The typical data are shown in Figure 1.

*Note:* The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

Lysyl Oxidase Fluorometric Assay Kit allows for the detection and quantification of endogenous Lysyl Oxidase concentrations within the range of 0.05ug - 3ug in cell lysates, sera, and plasma.
TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com or contact us at tech@immunoway.com

NOTES