Recombinant Human Lysyl Oxidase Homolog 3/LOXL3

Catalog Number: 6069-AO

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Lysyl Oxidase Homolog 3/LOXL3 protein Met1-Ile753, with a C-terminal 10-His tag Accession # P58215
N-terminal Sequence Analysis	Ser26
Structure / Form	Monomer
Predicted Molecular Mass	82 kDa

SPECIFICATIONS

SDS-PAGE	90-100 kDa, reducing conditions
Activity	Measured by its ability to produce hydrogen peroxide during the oxidation of benzylamine. The specific activity is >5 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Sodium Borate, 250 mM Urea, 10 mM $CaCl_2$, pH 8.0
- Recombinant Human Lysyl Oxidase Homolog 3/LOXL3 (rhLOXL3) (Catalog # 6069-AO)
- Coupling Enzyme: Horseradish Peroxidase (HRP) (250-330 U/mg) (Sigma, Catalog # P8375), 250 units/mL stock in 0.1 M Sodium Phosphate, pH 8.0
- Substrate Component 1: Benzylamine (Sigma, Catalog # B5136), 100 mM stock in deionized water
- Substrate Component 2: Amplex Ultra Red (AUR) (Molecular Probes, Catalog # A36006), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhLOXL3 to 40 ng/µL in Assay Buffer.
2. Dilute Benzylamine to 16 mM in Assay Buffer.
3. Combine equal volumes of 40 ng/µL rhLOXL3 and 16 mM Benzylamine. Also create a Substrate Blank by combining equal volumes of Assay Buffer and 16 mM Benzylamine.
4. Incubate the reactions for 30 minutes at 37 °C.
5. Prepare the Substrate Mixture containing 2 units/mL HRP and 40 µM AUR in Assay Buffer.
6. Load 50 µL of the incubated reactions into the wells of a black well plate, and add 50 µL of Substrate Mixture to each well.
7. Read at excitation and emission wavelengths of 544 nm and 590 nm (top read), respectively, in endpoint mode. Note: A cutoff must be set manually at a wavelength of 570 nm.
8. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Fluorescence* (RFU) x Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank.
**Derived using a fluorescent standard prepared by incubating 20 µM AUR, 1 unit/mL HRP, 4 mM Benzylamine, and a curve of Hydrogen Peroxide (Sigma, Catalog # H1009) in Assay Buffer. Use this oxidized AUR curve to determine the conversion factor.

Final Assay Conditions

Per Well:

- rhLOXL3: 1.0 µg
- Benzylamine: 4 mM
- HRP: 1 unit/mL
- AUR: 20 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

Lysyl oxidase-like protein 3 (LOXL3) is a secreted copper amine oxidase. The N-terminal region of LOXL3 contains four scavenger receptor cysteine-rich (SRCR) domains. The C-terminal region consists of a catalytic domain similar to other lysyl oxidases and a cytokine receptor-like domain (1). The catalytic domain contains conserved residues required for copper binding and formation of a lysyl tyrosylquinone cofactor (2). LOXL3 is expressed primarily in epidermis, kidney, aorta, and chondrocytes (3). The enzyme is expressed at low levels in most other tissues. Like lysyl oxidase, LOXL3 oxidizes the lysine ε-amino groups of a number of collagen substrates, and is also active against other primary amines (4). However, LOXL3 does not require proteolytic processing to be activated.

References:

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4. Lee, J. and Y. Kim (2006) J. Biol. Chem. **281**:37282.

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