Recombinant Cynomolgus Monkey/Rhesus Macaque Lysyl Oxidase Homolog 2/LOXL2 His-tag

Catalog Number: 11431-AO

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Lysyl Oxidase Homolog 2/LOXL2 protein Gln26-Gln774, with a C-terminal 6-His tag Accession # XP_005562900.1
N-terminal Sequence Analysis	Gln26 inferred from enzymatic pyroglutamate treatment revealing Tyr27
Predicted Molecular Mass	85 kD

SPECIFICATIONS

SDS-PAGE	92-102 kDa, under reducing conditions
Activity	Measured by its ability to produce hydrogen peroxide during the oxidation of benzylamine. The specific activity is >6 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, 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 Cyno/Rhesus Lysyl Oxidase Homolog 2 (rcynoLOXL2) (Catalog # 11431-AO)
- Coupling Enzyme: Horseradish Peroxidase (HRP), 250 units/mL stock in 0.1 M Sodium Phosphate, pH 8.0
- Substrate Component 1: Benzylamine, 100 mM stock in deionized water
- Substrate Component 2: Amplex Ultra Red (AUR), 10 mM stock in DMSO
- 96-Well Black Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rcynoLOXL2 to 20 µg/mL in Assay Buffer.
2. Dilute Benzylamine to 8 mM in Assay Buffer.
3. Combine equal volume of 20 µg/mL rcynoLOXL2 and 8 mM Benzylamine. Also create a Substrate Blank by combining equal volumes of Assay Buffer and 8 mM Benzylamine.
4. Incubate the reactions for 30 minutes at 37 °C.
5. Prepare 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 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 cut off must be set 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, 2 mM Benzylamine, and a curve of Hydrogen peroxide in Assay Buffer. Use this oxidized AUR curve to determine the conversion factor.

Final Assay Conditions

Per Well:

- rcynoLOXL2: 0.5 µg
- Benzylamine: 2 mM
- HRP: 1 unit/mL
- Amplex UltraRed: 20 µM

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Recombinant Cynomolgus Monkey/Rhesus Macaque Lysyl Oxidase Homolog 2/LOXL2 His-tag

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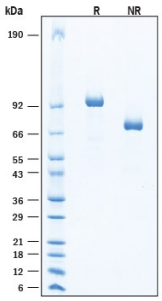
PREPARATION AND STORAGE

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.

DATA

SDS-PAGE



Recombinant Cynomolgus Monkey/Rhesus Macaque Lysyl Oxidase Homolog 2/LOXL2 His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Cynomolgus Monkey/Rhesus Macaque Lysyl Oxidase Homolog 2/LOXL2 His-tag Protein (Catalog # 11431-AO) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 92-102 kDa, under reducing conditions.

BACKGROUND

Lysyl Oxidase Homolog 2 (lysyl oxidase-like protein 2, LOXL2) is a member of a five-member lysyl oxidase-like (LOXL) gene family of secreted copper-binding amine oxidases that oxidize primary amine substrates such as collagen and elastin to aldehydes within the extracellular matrix (1, 2). LOXL2 is a secreted protein expressed in many tissues, with elevated levels in reproductive tissues such as placenta, uterus, and prostate (3). The N-terminal region of LOXL2 contains four scavenger receptor cysteine-rich (SRCR) domains responsible for protein-protein interactions while the C-terminal region contains the catalytic domain (1, 2). The catalytic domain contains conserved residues required for copper binding, a lysyl tyrosylquinone (LTQ) element required for co-factor formation essential for activity, and a cytokine receptor-like (CRL) domain (2,4). Modulation of the tissue microenvironment implicates a role for LOXL2 in many pathological conditions including fibrosis, atherosclerosis, and tumor development (5). LOXL2 has been shown to promote cell migration and tumor cell invasiveness (6,7) and to enhance tumor progression in various types of cancer including colon, gastric, hepatic, renal cancers (8,9). Dysregulation of LOXL2 has been linked to fibrosis and inflammation (10). Given its role in modulation of the tissue microenvironment involved in pathological conditions, development of inhibitors to LOXL2 is of interest for therapeutic application (2, 9).

References:

1. Csiszar, H. (2001) Prog. Nucleic Acid Res. Mol. Biol. **70**:1.
2. Radic, J. *et. al.* (2023) Int. J. Mol. Sci. **24**:11745.
3. Jourdan-Le Saux C. *et al.* (1999) J. Biol. Chem. **274**:12939.
4. Maki, J.M. and K.I. Kivirikko (2001) Biochem. J. **355**:381.
5. Wang, T.H. *et. al.* (2016) Int. J. Mol. Sci. **18**:62.
6. Akiri, G. *et al.* (2003) Cancer Res. **63**:1657.
7. Hollosi, P. *et al.* (2009) Int. J. Cancer. **125**:318.
8. Peinado, H. *et al.* (2008) Cancer Res. **68**:4541.
9. Liburkin-Dan, R. *et. al.* (2022) Int. J. Mol. Sci. **23**:6249.
10. Poe, A. *et. al.* (2023) Am. J. Physiol. Cell Physiol. **325**:C694.

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