Recombinant Mouse Lysyl Oxidase Homolog 2/LOXL2

Catalog Number: 9259-AO

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Gln26-Gln776 Accession # P58022 with a C-terminal 6-His tag
N-terminal Sequence Analysis	No results obtained. Gln26 inferred from enzymatic pyroglutamate treatment revealing Tyr27
Predicted Molecular Mass	85 kDa

SPECIFICATIONS

SDS-PAGE	93-111 kDa, reducing conditions
Activity	Measured by its ability to produce hydrogen peroxide during the oxidation of benzylamine. The specific activity is >4.5 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>80%, 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 Mouse Oxidase Homolog 2/LOXL2 (rmLOXL2) (Catalog # 9259-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 rmLOXL2 to 40 ng/µL in Assay Buffer.
2. Dilute Benzylamine to 8 mM in Assay Buffer.
3. Combine equal volumes of 40 ng/µL rmLOXL2 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 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, 2 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:

- rmLOXL2: 1 µg
- Benzylamine: 2 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.

bio-techne
bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956

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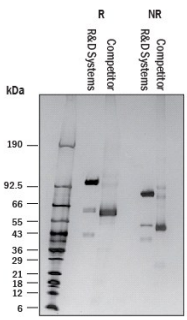
DATA

SDS-PAGE



1 µg/lane of Recombinant Mouse Lysyl Oxidase Homolog 2 (Catalog # 9259-AO) and 1 µg/lane of competitor Lysyl Oxidase Homolog 2 was resolved with 4-20% SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining. The competitor product is predominantly truncated/degraded material.

BACKGROUND

Lysyl Oxidase Homolog 2 (lysyl oxidase-like protein 2, LOXL2) is a member of lysyl oxidase-like (LOXL) gene family which includes LOXL1 through LOXL4. These enzymes are secreted copper-binding amine oxidases that oxidize primary amine substrates to aldehydes (1). The N-terminal region of LOXL2 contains four scavenger receptor cysteine-rich (SRCR) domains, and the C-terminal region is a catalytic domain similar to other lysyl oxidases (1). The catalytic domain contains conserved residues required for copper binding and formation of a lysyl tyrosylquinone co-factor (2). It has been shown that LOXL2 promotes cell migration and tumor cell invasiveness (3, 4). Elevated expression of LOXL2 is also associated with cancer progression in various tumors and carcinoma cell lines, which makes it a potential marker for prognosis of cancer (5). LOXL2 is expressed in many tissues, with elevated levels in reproductive tissues such as placenta, uterus, and prostate (6). Activity is routinely determined using benzylamine as a substrate, although significantly higher activity is detected when activity is quantified using lysine as the substrate.

References:

1. Csiszar, H. (2001) Prog. Nucleic Acid Res. Mol. Biol. **70**:1.
2. Maki, J.M. and K.I. Kivirikko (2001) Biochem J. **355**:381.
3. Akiri, G. *et al.* (2003) Cancer Res. **63**:1657.
4. Hollosi, P. *et al.* (2009) Int. J. Cancer. **125**:318.
5. Peinado, H. *et al.* (2008) Cancer Res. **68**:4541.
6. Jourdan-Le Saux C. *et al.* (1999) J. Biol. Chem. **274**:12939.

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bio-techne.com
Global info@bio-techne.com techsupport@bio-techne.com
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373
Rest of World bio-techne.com/find-us/distributors TEL +1 612 379 2956