

HAO1 - Human Hydroxyacid Oxidase 1

PDB:2NZL

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:NM_017545

Entry Clone Source:Origene

SGC Clone Accession:

Tag:N-terminal hexahistidine tag, mhhhhhssgvdlgtenlyfqs

Host:BL21(DE3)-R3/Rosetta

Construct

Prelude:

Sequence:

mhhhhhssgvdlgtenlyfqsMLPR LIC INDYEQHAKSVLPKSIYDYRSGANDEET LADNIAAFSRWKLYPRMLRNVAETDLS
TS VLGQRVSMPICVGATAMQRM AHVDGELAT VRACQSLGTGMM LSSWATSSIEEVAEAGP EALRWLQLYIYKDREVTKKLV RQA
EKMGY KAIFVTVDTPYLG NR LDDVRNRFKLPPQL RMKNFETSTLSFSPEENFGDDSGLAAYVA KAIDPSISWEDIKWLRR L TSL
PIVAKGIL RGDDAREAVKHGLNGILVSNHGARQLDGV PATIDVLPEIVEAVEGKVEVFLDGGVRKG TDVLKALALGAKAVFVGR
PIVWGLAFQGE KGVQDVLEILKEEFRLAMALSGCQNVKVI DKTLVRKNPLAVSKI

Vector:pNIC28-Bsa4

Growth

Medium:

Antibiotics:

Procedure:An overnight culture (20 ml) was used to inoculate 1L TB with 50 µg/ml of Kanamycin. The cells were cultured at 37°C until the OD reached 4.0 and then the temperature was decreased to 25°C. IPTG was added to 0.5 mM final concentration and the culture kept at 25°C overnight. Cells were harvested by centrifugation and the pellet stored at -80°C.

Purification

Procedure

Extraction

Procedure

The cell pellet from 1 L of culture was resuspended in 40ml of extraction buffer. The sample was homogenized by passing once through the Constant cell systems fluidizer, the DNA precipitated by adding 0.15% PEI and incubated on ice for 45mins and then centrifuged at 37000 xg. The supernatant was filtered through 0.2 m M serum acrodiscs and kept for further purification.

Concentration:

Ligand

MassSpec: The mass was verified by Mass spec to be correct within 1Da

Crystallization: Crystals were grown by vapor diffusion at 20°C. A sitting drop consisting of 50 nl of protein (12 mg/ml) and 100 nl well solution was equilibrated against well solution containing 30% PEG 1000, 0.1 M Malate-MES-Tris pH 7.0. The crystal was transferred to a cryo-protectant comprised of well solution supplemented with 20% ethylene glycol before flash-cooling in liquid nitrogen.

NMR Spectroscopy:

Data Collection: Resolution: 1.35 Å, X-ray source: Synchrotron SLS-X10SA, single wavelength.

Data Processing: