ALDH7A1

PDB:2J6L

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC002515
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence mhhhhhhssgvdlgtenlyfq*s
Host:BL21(DE3)-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqSMSTLLIN QPQYAWLKELGLREENEGVYNGSWGGRGE VITTYCPANNEPIARVRQASVADYEET
VK KAREAWKIWADIPAPKRGEIVRQIGDALR EKIQVLGSLVSLEMGKILVEGVGEVQEYV DICDYAVGLSRMIGGPILPSERSG
HALIE QWNPVGLVGIITAFNFPVAVYGWNNAIAM ICGNVCLWKGAPTTSLISVAVTKIIAKVL EDNKLPGAICSLTCGGADIGT
AMAKDERV NLLSFTGSTQVGKQVGLMVQERFGRSLLE LGGNNAIIAFEDADLSLVVPSALFAAVGT AGQRCTTARRLFIHESIH
DEVVNRLKKAY AQIRVGNPWDPNVLYGPLHTKQAVSMFLG AVEEAKKEGGTVVYGGKVMDRPGNYVEPT IVTGLGHDASIAHTE
TFAPILYVFKFQNE EEVFAWNNEVKQGLSSSIFTKDLGRIFRW LGPKGSDCGIVNVNIPTSGAEIGGAFGGE KHTGGGRESGSD
AWKQYMRRSTCTINYS

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: An overnight culture (10 ml) was used to innoculate 1L TB with 50 µg/ml of Kanamycin (total 6L). The cells were cultured at 37°C until the OD reached 0.65 and then the temperature was decreased to 18°C. IPTG was added to 0.5 mM final concentration and the culture kept at 18°C overnight.

Purification

Procedure
Column 1: Ni-Sepharose
The column was packed with 2 ml of Ni-Sepharose slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the flow through was collected. The

column was washed with 50 ml of binding buffer and then 50 ml of washing buffer. The protein was eluted with 12 ml of elution buffer and collected in 1.5 ml fractions.

Column 2: Superdex 200 Hiload 16/60
Procedure: An AKTA Purifier system was used. The fractions were analyzed by SDS - PAGE and combined together for TEV cleavage.

Enzymatic treatment : 300 µl of TEV protease were added into the the sample after gel filtration. The sample was incubated at 4°C overnight

Column 3: Ni - Sepharose
Procedure: After treatment with TEV protease, the sample was loaded onto the column (packed with 0.5 ml of Ni-Sepharose slurry). The flow through was collected and the column was then washed with 3 ml of the buffer (also collected).

Concentration: The protein was concentrated in Amicon to 38.8 mg/ml.

Extraction

Procedure
The cells were harvested by centrifugation at 4,000 g for 10 min. The pellet from 1 L of culture was resuspended in 25 ml of extraction buffer. The sample was homogenized by using the EmulsiFlex-05 homogenizer (Glen Creston) and then centrifuged at 37000 xg. The supernatant was kept for further purification.
Concentration:
Ligand
MassSpec: The experimentally determined mass of 54115 agrees well with the expected mass of 54113.8.
Crystallization: Crystals were grown by vapour diffusion at 20°C in 300 nl sitting drops. NADH to a final concentration of 11 mM was added to the protein just prior to crystallisation. The drops were prepared by mixing 150 nl of protein solution (30 mg/ml) and 150 nl of buffer consisting of 0.2 M NaBr , 0.1 M Bis-Tris Propane pH 6.5 and 20% PEG 3350 and 10% EtGly. Crystals were transferred to a cryo-protectant consisting of 20% glycerol, 80 % well solution before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: