KIAA0089

PDB:2PLA

Revision

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Revised by:created
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Entry Clone Accession:gi|24307999
Entry Clone Source:MGC
SGC Clone Accession:KIAA0089A-c002
Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence
mhhhhhhssgvdlgtenlyfq*s
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfq*sMAAAPLKVCIVGSGNWGSAVAKIIGNNVKKLQKFASTVKMWVFEETVNGRKLTDIINNDHENVK
YLPGHKLPENVVAMSNLSEAVQDADLLVFVIPHQFIHRICDEITGRVPKKALGITLIKGIDEGPEGLKLISDIIREKMGIDISVLMG
ANIANEVAAEKFCETTIGSKVMENGLLFKELLQTPNFRITVVDDADTVELCGALKNIVAVGAGFCDGLRCGDNTKAAVIRLGLMEMI
AFARIFCKGQVSTATFLESCGVADLITTCYGGRNRRVAEAFARTGKTIEELEKEMLNGQKLQGPQTSAEVYRILKQKGLLDKFPLFT
AVYQICYESRPVQEMLSCLQSHPE
```

Vector:pNIC28-Bsa4.

Growth

Medium:TB
Antibiotics:
Procedure: An overnight culture (10 ml) was used to innoculate 1L TB medium (supplemented with 50 µg/ml of Kanamycin). The cells were cultured in 2 x 1 litre at 37°C with vigorous shaking (160 rpm) until the culture reached an OD600 of 1.5. At that point temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and cultured further for 18 hours. Cells were harvested at 6000 rpm for 10 minutes and the cell pellet of 1L was resuspended in 20 ml of lysis buffer and stored at -20°C until further use.

Purification

Procedure
Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)
Column 2: Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)
AKTA Xpress Affinity/Gel Filtration . The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10

volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280nm was automatically collected.
AKTA Xpress Affinity/Gel Filtration. The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 0.80 ml/min. Eluted protein was collected in 2 ml fractions in a 96well block.

Extraction

Procedure
The resuspended pellet was thawed and homogenised by using Emulsiflex-C5 homogenizer (Avestin) 5x and then centrifuged at 4 °C in Beckman JA-17 rotor at 16000 rpm for 45 min.
Concentration: The protein was concentrated to 22.8mg/ml by using an Amicon Ultra 10k concentrator (Millipore).
Ligand
MassSpec: The experimentally determined mass of KIAA0089A was 40733Da, which corresponds to the theoretical mass.
Crystallization: Crystals were grown by vapor diffusion in sitting drops at 4°C. Before setting up the experiment NADH was added to the protein to a final concentration of 5 mM. A sitting drop consisting of 100 nl protein and 50 nl well solution was equilibrated against well solution containing 50% PEG 300, 0.2 M NaCl, 0.1 M sodium/potassium phosphate pH 6.2. The crystal was mounted in a loop directly from the drop and flash-cooled in liquid nitrogen.
NMR Spectroscopy:
Data Collection:Resolution: 2.50 Å; X-ray source: Beamline SLS -X10SA, single wavelength.
Data Processing: