Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5188183

SGC Construct ID: ACBD4A-c010

GenBank GI number: gi|13376029

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Amplified construct sequence:
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGAGCCCAGAGCCCGAC
TGCCAGAAACAGTTCCAGGCTGCAGTGAG
CGTCATCCAGAACCTGCCCAAGAACGGTT
CTTACCGCCCCTCCTATGAAGAGATGCTG
CGATTCTACAGTTACTACAAGCAGGCCAC
CATGGGGCCCTGCCTGGTCCCCCGGCCCG
GGTTCTGGGACCCCATTGGACGATATAAG
TGGGACGCCTGGAACAGTCTGGGCAAGAT
GAGCAGGGAGGAGGCCATGTCTGCCTACA
TCACTGAAATGAAACTGGTGGCACAGAAG
GTGATCGACACAGTGCCCCTGGGTGAGGT
GGCAGAGTGACAGTAAAGGTGGATACGGA
TCCGAA

Tags and additions: N-terminal Histidine-tag with TEV protease cleavage site.

Final protein sequence (tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfqsMSPEPDC
QKQFQAAVSVIQNLPKNGSYRPSYEEMLR
FYSYYKQATMGPCLVPRPGFWDPIGRYKW
DAWNSLGKMSREEAMSAYITEMKLVAQKV
IDTVPLGEVAE

Host: BL21(DE3)-R3 pRARE2

Growth medium, induction protocol: 10 µl of a glycerol stock was inoculated into 5ml of TB medium (supplemented with 50 µg/ml Kanamycin, 34 µg/ml Chloramphenicol) and cultured at 37°C overnight in a shaking incubator (275 rpm). Next day 0.75 ml of overnight culture was used to inoculate 1 litre of TB medium (6 x) and grown at 37°C with vigorous shaking (160 rpm) until the culture reaches an OD_{600} of 1.5. Temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and further cultivated for 16 hrs. Cells were harvested by centrifugation at 6500 rpm for 10 min, and the cell pellet was stored at -20°C until further use.

Extraction buffer, extraction method: Lysis buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole, Complete® protease inhibitors (Roche, 1 tbl/50 ml). Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by using sonicator, and a supernatant containing the target protein was obtained by centrifugation at 21,000 (rpm) for 45 minutes.

Column 1: Ni-Sepharose 6 Fast Flow

Buffers: Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole; **Wash buffer:** 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM Imidazole; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole. **Note:** All the buffers contain 0.5mM TCEP.

Procedure: The column was packed with 2 ml of Ni-Sepharose 6 Fast Flow slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the column was washed with 20

ml of binding buffer and then 20 ml of washing buffer. The protein was eluted with 10 ml of elution buffer.
Column 2: SuperDex 75 16/60 HiLoad (GE/Amersham).
Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.
Procedure: The eluted protein from the Ni-affinity column was loaded on the gel filtration column in GF buffer at 1.0 ml/min on an AKTA Purifier system. Eluted proteins were collected in 1 ml fractions.
Enzymatic treatment: TEV cleaved.
Column 3: Ni-NTA (TEV clean up)
Buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP
Procedure: Total 5 mgs of protein was cleaved with 300 ug of TEV protease at 4 degree for 48 hours.
TEV clean up: The TEV cleaved protein was applied to a 1 ml Ni-NTA column, already equilibrated with gel filtration buffer (10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP). The flow through form the column was collected. The eluate from the column was monitored by SDS gel analysis.
Concentration: The target protein was concentrated to 15.5 mg/ml using Vivaspin 5K concentrators and stored at -80°C.
Mass spectrometry characterization: Corresponds to theoretical mass, as determined by ESI-TOF MS.
Crystallization: Crystals were grown by vapour diffusion in sitting drops at 4°C. Before setting up the experiment stearoyl-CoA was added to the protein to a final concentration of 2mM. A sitting drop consisting of 120 nl protein and 40 nl well solution was equilibrated against well solution containing 0.24M K thiocyanate; 26w/v PEG3350. Crystals were cryo protected in 25% ethylene glycol and flash-cooled in liquid nitrogen.
Data Collection, Resolution: 2.1 Å**, X-ray source:** Synchrotron SLS-X10SA, single wavelength.