Entry Clone Source: MGC

Entry Clone Accession: IMAGE:6268667

SGC Construct ID: MGC5987A-c002

GenBank GI number: gi|33457311

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

Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGGCGCGGCTACCGAAG
CTGGCAGTCTTTGATTTGGATTACACTCT
CTGGCCTTTCTGGGTCGACACGCACGTAG
ACCCTCCGTTCCATAAGAGCAGTGATGGA
ACTGTACGAGATAGGCGGGGCCAAGACGT
CCGACTGTACCCAGAGGTGCCTGAGGTCC
TAAAACGATTGCAGAGCCTTGGGGTGCCC
GGTGCGGCTGCTTCAAGGACAAGTGAGAT
AGAAGGGGCCAACCAGCTACTGGAGCTCT
TTGACCTCTTCAGGTACTTTGTTCATCGG
GAAATCTATCCAGGCAGCAAGATCACACA
CTTTGAGAGGTTGCAGCAGAAGACTGGAA
TTCCTTTCTCCCAGATGATCTTCTTTGAT
GATGAGAGGCGGAATATTGTAGACGTCAG
CAAACTGGGTGTTACCTGCATTCACATCC
AGAATGGAATGAATCTTCAAACTCTAAGT
CAAGGGTTAGAGACATTTGCGAAGGCCCA
AACTGGGCCTTTGTGACAGTAAAGGTGGA
TACGGATCCGAA
```

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

Final protein sequence (Tag sequence in lowercase):

```
mhhhhhhssgvdlgtenlyfqsMARLPKL
AVFDLDYTLWPFWVDTHVDPPFHKSSDGT
VRDRRGQDVRLYPEVPEVLKRLQSLGVPG
AAASRTSEIEGANQLLELFDLFRYFVHRE
IYPGSKITHFERLQQKTGIPFSQMIFFDD
ERRNIVDVSKLGVTCIHIQNGMNLQTLSQ
GLETFAKAQTGPL
```

Host: BL21(DE3)-R3 pRARE2

Growth medium, induction protocol: 10 µl of a glycerol stock was inoculated into 5ml of TB medium (supplemented with 50µg/ml Kanamycin, 34µg/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, 300 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: none.
Concentration: The target protein was concentrated to 5.1 mg/ml using Vivaspin 10K concentrators and stored at -80C.
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 $MgCl_2$ was added to the protein to a final concentration of 1mM. A sitting drop consisting of 100 nl protein and 50 nl well solution was equilibrated against well solution containing 0.2M Na_3(cit), 20% PEG 3350. Crystals were cryo protected in 20% ethylene glycol and flash-cooled in liquid nitrogen.
Data Collection, Resolution: 2.0 Å**; X-ray source:** FREL, single wavelength.