Entry Clone Source: Stephan Feller Oxford University

Entry Clone Accession: n/a

SGC Construct ID: IQSEC1A-c001

GenBank GI number: gi|197304786

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

Amplified construct sequence:
CATATGCACCATCATCATCATCATTC
TTCTGGTGTAGATCTGGGTACCGAGA
ACCTGTACTTCCAATCCATGGGCTGT
GTGCTCTCTCTGCCCCACCGTCGGTT
GGTCTGCTACTGCCGGCTCTTTGAGG
TTCCAGACCCAAACAAGCCCCAGAAA
CTCGGACTACACCAGCGAGAAATCTT
CCTGTTCAACGACCTCCTGGTGGTCA
CCAAGATCTTCCAGAAGAAGAAGAAC
TCGGTGACGTACAGCTTCCGACAGTC
CTTCTCCTTGTACGGCATGCAGGTCC
TGCTCTTCGAGAACCAGTACTACCCC
AATGGCATCCGGCTCACCTCGTCTGT
CCCCGGAGCAGATATCAAAGTGTTAA
TAAACTTCAACGCCCCCAACCCTCAA
GACCGGAAGAAATTCACCGATGACCT
GCGGGAGTCCATTGCGGAAGTCCAAG
AGATGGAGAAGCACAGGATAGAGTCG
GAGCTCGAGAAGCAGAAATGACAGTA
AAGGTGGATACGGATCCGAA

Final protein sequence (Tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfq^smGC
VLSLPHRRLVCYCRLFEVPDPNKPQK
LGLHQREIFLFNDLLVVTKIFQKKKN
SVTYSFRQSFSLYGMQVLLFENQYYP
NGIRLTSSVPGADIKVLINFNAPNPQ
DRKKFTDDLRESIAEVQEMEKHRIES
ELEKQK

^ TEV cleavage site

Tags and additions: Cleavable N-terminal His6 tag.

Host: BL21 (DE3)R3-pRARE2 (Phage resistant strain).

Growth medium, induction protocol: 10ml from a 50ml overnight culture containing 50µg/ml kanamycin and 34µg/ml chloramphenicol were used to inoculate each of two 1L cultures of TB containing 50µg/ml kanamycin and 34µg/ml chloramphenicol. Cultures were grown at 37°C until the OD_{600} reached ~2.5 then the temperature was adjusted to 18°C. Expression was induced overnight using 0.1 mM IPTG at an OD_{600} of 3.0. The cells were collected by centrifugation and the pellet re-suspended in binding buffer and frozen.
Lysis buffer: 20 mM TRIS, pH 8.5; 500 mM NaCl; 5 mM Imidazole; 5% Glycerol.

Extraction buffer, extraction method: Frozen pellets were thawed and fresh 0.5 mM TCEP, 1 mM PMSF added to the lysate. Cells were lysed by high pressure cell disrupter. The lysate was centrifuged at 17,000rpm for 60 minutes and the supernatant collected for purification.

Column 1: Ni-affinity. Ni-sepharose (Amersham), 5ml of 50% slurry in 1.5x10cm column, washed with binding buffer.

Column 1 Buffers:
Binding buffer: 20 mM TRIS, pH 8.5; 500 mM NaCl; 5% glycerol; 5 mM Imidazole.
Wash buffer: 20 mM TRIS, pH 8.5; 500 mM NaCl; 5% glycerol; 30 mM Imidazole.
Elution buffer: 20 mM TRIS, pH 8.5; 500 mM NaCl; 5% glycerol; 50 to 400 mM Imidazole.

Column 1 Procedure: The supernatant was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 30ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5ml portions of elution buffer with increasing concentration of imidazole (50, 100, 150, 200, 250 and 400 mM) fractions were collected until essentially all protein was eluted.

Enzymatic treatment: The N-terminal His tag was cleaved by treatment with TEV protease, overnight.

Column 2: Size Exclusion Chromatography. Superdex S75 16/60 HiLoad.

Column 2 Buffer: 20 mM TRIS, pH 8.5; 300 mM NaCl; 5% glycerol.

Column 2 Procedure: The protein was concentrated and applied to an S75 16/60 HiLoad gel filtration column equilibrated in 20 mM TRIS pH 8.5, 300 mM NaCl, 5% glycerol using an ÄKTA express system

Mass spectrometry characterization: LC- ESI -MS TOF gave a measured mass of 16409Da for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 3.7mg/ml using an Amicon 10kDa cut-off concentrator.

Crystallisation: Crystals grown at 4°C using a protein solution (3.7mg/ml) and a reservoir solution containing 17.5% PEG3350, 0.2 M $NaNO_3$, 10% Ethylene glycole, 1.0 M BTProp pH 8.25.

Data collection: Crystals were cryo-protected using the well solution supplemented by 20% ethylene glycol and flash frozen in liquid nitrogen.
X-ray source: Diffraction data were collected from a single crystal on a Diamond beamline IO3, the structure was refined to 2.06Å.