Entry Clone Source: MGC

Entry Clone Accession: BC029424

SGC Construct ID: LOC493869A-c008

GenBank GI number: gi|56606000

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

Tags and additions: TEV-cleavable (*), N-terminal histag. Tag sequence:
mhhhhhhssgvdlgtenlyfq*sm.

Protein sequence:
mhhhhhhssgvdlgtenlyfqsmINSFYA
FEVKDAKGRTVSLEKYKGKVSLVVNVASD
CQLTDRNYLGLKELHKEFGPSHFSVLAFP
CNQFGESEPRPSKEVESFARKNYGVTFPI
FHKIKILGSEGEPAFRFLVDSSKKEPRWN
FWKYLVNPEGQVVKFWRPEEPIEVIRPDI
AALVRQVIIKKKEDL

Host: BL21(DE3)-R3-pRARE2

Growth medium, induction protocol: Medium: TB + 50 µg/ml Kanamycin + 34 µg/ml chloramp. 2 x1 liter TB in 3-L flasks were inoculated with 10 ml overnight culture and grown at 37°C. The protein expression was induced with 0.5 mM IPTG at OD_{600} = 2.5 at 18°C over night. The cells were collected by centrifugation and frozen at -80°C.

Extraction buffer, extraction method: Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% glycerol, 0.5 mM TCEP, Complete® protease inhibitors (1 tablet/50 ml) and 5 U/ml of Benzonase. Cell pellets were resuspended in a total volume of 50 ml lysis buffer. The cells were disrupted by high pressure (20 kpsi) and nucleic acids and cell debris removed by adding 0.15% PEI, followed by centrifugation for 30 minutes at 40,000xg. The supernatant was further clarified by filtration (0.45 µm).

Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)

Buffers: Lysis buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% glycerol, 0.5 mM TCEP and 5 U/ml of Benzonase; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 40 mM Imidazole, 5% glycerol and 0.5 mM TCEP; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM Imidazole, 5% glycerol 0.5 mM TCEP.

Procedure: 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.

Column 2: Hiload 16/60 Superdex 75 prep grade 120 ml (GE/Amersham Biosciences)

Buffers: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol

Procedure: The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column at 0.80 ml/min. Eluted proteins were collected in 2 ml fractions.

Concentration: The protein was concentrated in Amicon (5 K) to 28 mg/ml. The protein concentration was determined spectrophotometrically using the predicted molar extinction coefficient 25440($M^{-1}cm^{-1}$).

Mass spec characterization: The mass determined for LOC493869A-p003 was 21849.2 Da, in agreement with the predicted mass of the his-tagged protein.

Crystallisation: Crystals were grown by vapor diffusion at 20°C from a sitting drop consisting of 200 nl protein (28 mg/ml) and 400 nl well solution. The drop was equilibrated against well solution containing 0.1 M ammonium sulfate, 2.5 % PEG 400 and 0.1 M. HEPES pH 6.8. The crystal was transferred to a cryoprotectant composed of 25 % ethylene glycol before flash-cooling in liquid nitrogen.

Data Collection: Resolution: 2.05Å. **X-ray source:**Synchrontron SLS-X10.