Entry Clone Source: n/a

Entry Clone Accession: GI:34189907

SGC Construct ID: PCCAA-c002

GenBank GI number: gi|65506442

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

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

Host: BL21(DE3)-R3-pRARE2

Sequence (tag sequence in lower case) :
mhhhhhhssgvdlgtenlyfqs*mTSSVL
RSPMPGVVVAVSVKPGDAVAEGQEICVIE
AMKMQNSMTAGKTGTVKSVHCQAGDTVGE
GDLLVELE

Expression: Rosetta cells were grown in 1x TB medium supplemented with 50 µg/ml kanamycin at 37°C. Cells were induced at an optical density of 0.9 – 1.0 with 0.1 M IPTG at 18°C. 20 hours post-induction the cultures were collected and centrifuged for 30min at 15000rpm. The pellet was resuspended in lysis buffer (50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml)) and frozen at -20°C until purification.

Extraction: Lysis buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml). The thawed cellular lysates were supplemented with benzonase (Novagen) (25u/10ml of lysate). Cells were lysed with sonication (20 minutes, 5 sec pulse on, 5 sec cooling), followed by centrifugation for 60 min at 15000rpm at 4°C.

Column 1: Ni-affinity, Ni-Sepharose - (GE Healthcare) purification in batch

Buffers: Start buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol, 0.5mM TCEP; **Washing buffer:** 50mM HEPES pH 7.5, 500mM NaCl, 20mM Imidazole, 5% glycerol, 0.5mM TCEP; **Elution buffer:** 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 250mM Imidazole.

Procedure: The cell extract was incubated with Ni-Sepharose (5 mL of resin/lysate from 2 l of culture) during 1h at 4°C with gentle rotation. The resin was centrifuged at 1000g for 5min at 4°C, supernatant was removed and the resin was loaded on the gravity column. Resin was washed 4x with 50ml of washing buffer. Protein was eluted in 10 elution fractions (2 ml each). Protein fractions were analysed by SDS-PAGE. Target protein containing fractions were concentrated and buffer was exchanged into lysis buffer using Amicon Ultra-15 concentrators with 3kDa cut-off. Protein was digested overnight with TEV (60 µg/mg target protein). TEV and His-tag were removed by mixing with 50 µL Ni-resin for 1 hour, and washing resin 3x 2mL with lysis buffer.

Concentration and buffer exchange: Using PD10 desalting columns, the buffer was exchanged into 50mM HEPES, 100mM NaCl, pH 7.5. The sample was concentrated to 21mg/ml. Concentrations were determined from the absorbance at 280 nm using a NanoDrop spectrophotometer.

Mass spec characterization: The experimental mass coincides with th theoretical mass (7463Da).

Crystallization: Crystals were grown by vapor diffusion at 20°C in 150nl sitting drops. The drops were prepared by mixing 100nl of protein solution and 50nl of precipitant consisting of 20% PEG3350, 0.2M sodium formate. Crystals were flash-cooled in liquid nitrogen with 25 % ethylene glycerol as cryoprotectant.

Data Collection: Resolution: 1.5Å. **X-ray source:** Swiss Light source (SLS), beamline X-10.