GI number: gi|148612882

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

Amplified construct sequence:
CATATGCACCATCATCATCATCATTCTTCT
GGTGTAGATCTGGGTACCGAGAACCTGTAC
TTCCAATCCATGCGTGAAGAAAAAAAAACC
AAAGATCTGTTTGAACTGGATGATGATTTT
ACCGCCATGTATAAAGTGCTGGATGTGGTG
AAAGCCCACAAAGATAGCTGGCCGTTTCTG
GAACCGGTGGATGAAAGCTATGCGCCGAAT
TATTATCAGATCATTAAAGCGCCGATGGAT
ATTAGCAGCATGGAAAAAAAACTGAATGGT
GGCCTGTATTGCACCAAAGAAGAATTTGTG
AACGATATGAAAACCATGTTTCGTAACTGC
CGTAAATATAATGGTGAAAGCAGCGAATAT
ACCAAAATGAGCGATAACCTGGAACGTTGC
TTTCATCGTGCGATGATGAAACATTGACAG
TAAAGGTGGATACGGATCCGAA

Final protein sequence:
mhhhhhhssgvdlgtenlyfq^SMREEKKTKDLF
ELDDDFTAMYKVLDVVKAHKDSWPF
LEPVDESYAPNYYQIIKAPMDISSMEK
KLNGGLYCTKEEFVNDMKTMFRNCR
KYNGESSEYTKMSDNLERCFHRAMM
KH

^ TEV cleave 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 1 liter 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 1°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.
Binding buffer: 50 mM HEPES pH 7.5; 500 mM NaCl; 10 mM imidazole, 5% glycerol.
Extraction buffer, extraction method: Frozen pellets were thawed and fresh 0.5 µM TCEP, 1 mM PMSF added to the lysate. Cells were lysed using a French press. The lysate was centrifuged at 17,000 rpm for 60 minutes and the supernatant was collected for purification.

Column 1: Ni-affinity. Ni-sepharose (Amersham), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Column 1 Buffers:
Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 5% glycerol.
Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol.
Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 50 to 250 mM Imidazole (step elution).

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 mM, 100 mM, 150 mM, 200 mM and 250 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: 10 mM HEPES, pH 7.5; 500 mM NaCl, 5% glycerol.

Column 2 Procedure: The protein was concentrated and applied to an S75 16/60 HiLoad gel filtration column equilibrated in 10 mM HEPES, pH 7.5; 500mM NaCl, 5 % glycerol using an ÄKTAexpress system.

Mass spectrometry characterization: LC- ESI -MS TOF gave a measured mass of 13834 for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 10.4 mg/ml using an Amicon 3 kDa cut-off concentrator.

Crystallisation: NMP was added to the protein solution to a final concentration of 2 mM prior to crystallization. Crystals were grown at 4°C in 300 nl sitting drops from a 2:1 ratio of protein to reservoir solution containing 0.1 M MES pH 6.5 and 12% PEG 20,000.

Data Collection: Crystals were cryo-protected using the well solution supplemented by 30% sucrose and 20% ethylene glycol and flash frozen in liquid nitrogen.
X-ray source: Diffraction data were collected from a single crystal on a Rigaku FRE Superbright at a single wavelength of 1.5 Å and the structure was refined to 1.83 Å.
Phasing: The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.