Entry Clone Source: MGC

Entry Clone Accession: n/a

SGC Construct ID: BRD1A-c004

GenBank GI number: gi|11321642

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

Amplified construct sequence:

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CATATGCACCATCATCATCATCATTC
TTCTGGTGTAGATCTGGGTACCGAGA
ACCTGTACTTCCAATCCATGGAGCAG
GTCGCCATGGAGCTGCGGCTGACCCC
GCTGACGGTGCTGCTGCGCTCAGTGC
TGGACCAGCTGCAAGACAAGGACCCC
GCCAGGATATTTGCGCAGCCCGTGAG
TCTGAAGGAGGTACCAGATTATTTGG
ATCACATTAAACATCCCATGGACTTT
GCCACAATGAGGAAACGGTTAGAAGC
TCAAGGGTATAAAAACCTCCATGAGT
TTGAGGAGGATTTTGATCTCATTATA
GATAACTGCATGAAGTACAATGCCAG
GGACACCGTGTTCTATAGAGCCGCGG
TGAGGCTGCGCGATCAGGGAGGTGTT
GTTCTGAGGCAGGCCCGGCGCGAGGT
GGACAGCATCGGCTTGGAAGAGGCCT
CGGGGATGCACCTGCCTGAGCGGCCT
GCTTGACAGTAAAGGTGGATACGGAT
CCGAA
```

Final protein sequence (Tag sequence in lowercase):

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mhhhhhhssgvdlgtenlyfq^smEQ
VAMELRLTPLTVLLRSVLDQLQDKDP
ARIFAQPVSLKEVPDYLDHIKHPMDF
ATMRKRLEAQGYKNLHEFEEDFDLII
DNCMKYNARDTVFYRAAVRLRDQGGV
VLRQARREVDSIGLEEASGMHLPERP
A
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^ 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 50 ml overnight culture containing 50µg/ml kanamycin and 34µg/ml chloramphenicol were used to inoculate each of two 1L cultures of LB 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: 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 mM TCEP, 1 mM PMSF added to the lysate. Cells were lysed by sonication. 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: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 0.5 mM Imidazole.
Wash buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 30 mM Imidazole.
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 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, 500 mM NaCl, 5% glycerol using an ÄKTAexpress system.

Mass spectrometry characterization: LC- ESI -MS TOF gave a measured mass of 15640Da for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 17.3mg/ml using an Amicon 3kDa cut-off concentrator.

Crystallisation: Protein buffer was exchanged to 10 mM HEPES pH 7.5 and 300 mM NaCl. Crystals were grown at 4°C in 300nl sitting drops from a 2:1 ratio of protein to reservoir solution containing 0.1 M acetate pH 4.6, 32.5% PEG3350, 5% EtGly.

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 Diamond beamline I03 at a single wavelength of 1.00Å and the structure was refined to 2.06Å.
Phasing: The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.