Entry Clone Source: Synthetic

Entry Clone Accession: n/a

SGC Construct ID: WDR9A-c080

GenBank GI number: gi|16445436

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

Amplified construct sequence:

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CATATGCACCATCATCATCATCATTC
TTCTGGTGTAGATCTGGGTACCGAGA
ACCTGTACTTCCAATCCATGGCGACC
AACTATGTGGAAAGCAACTGGAAAAA
ACAGTGCAAAGAACTGGTGAACCTGA
TTTTTCAGTGCGAAGATAGCGAACCG
TTTCGTCAGCCGGTTGATCTGGTGGA
ATATCCGGATTATCGTGATATCATTG
ATACCCCGATGGATTTTGGCACCGTG
CGCGAAACCCTGGATGCCGGCAACTA
TGATAGCCCGCTGGAATTTTGCAAAG
ATATTCGCCTGATTTTTAGCAACGCG
AAAGCCTATACCCCGAACAAACGCAG
CAAAATCTATAGCATGACCCTGCGTC
TGAGCGCGCTGTTTGAAGAAAAAATG
AAAAAAATTAGCAGCGATTTTAAAAT
TGGTCAGAAATTTAACGAATGACAGT
AAAGGTGGATACGGATCCGAA
```

Final protein sequence (Tag sequence in lowercase):

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mhhhhhhssgvdlgtenlyfq^smAT
NYVESNWKKQCKELVNLIFQCEDSEP
FRQPVDLVEYPDYRDIIDTPMDFGTV
RETLDAGNYDSPLEFCKDIRLIFSNA
KAYTPNKRSKIYSMTLRLSALFEEKM
KKISSDFKIGQKFNE
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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 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.
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 mM TCEP, 1 mM PMSF added to the lysate. Cells were lysed using sonication. The lysate was centrifuged at 17,000 rpm for 60 minutes and the supernatant 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 Buffer:
Binding buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% glycerol; 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 proetin 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.

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

Column 2 Buffers: 10 mM HEPS, pH 7.5; 500 mM NaCl; 5% glycerol.

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

Mass spectrometry characterization: LC- ESI -MS TOF gave a measured mass of 14429 Da for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 9.6mg/ml using an Amicon 3kDa cut-off concentrator.

Crystallisation: Crystals were grown at 4°C in 150nl sitting drops from a 2:1 ratio of protein to reseroir solution containing 0.2M $(NH_4)OOCCH_3$, 0.1M bis tris pH 5.5 and 25% PEG3350.

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 bealine I02 at a single wavelength of 0.97Å. The final structure was refined to 1.66Å.
Phasing: The structure was solved by molecular replacement using an ensemble of known bromodomain structure as a starting model.