

Entry Clone Source: FivePrime
Entry Clone Accession: NM_058243 Variant
SGC Construct ID: BRD4A-c002
GenBank GI number: gi 19718731
Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]
GI number: gi 19718731
Amplified construct sequence: CATATGCACCATCATCATCATCATTCTTCT GGTAGATCTGGTACCGAGAACCTGTAC TTCCAATCCATGAACCCCCGGCCCCAGAG ACCTCCAACCCTAACAGCCAAAGAGGCAG ACCAACCAACTGCAATACCTGCTCAGAGTG GTGCTCAAGACACTATGGAAACACCAGTTT GCATGGCCTTCCAGCAGCCTGTGGATGCC GTCAAGCTGAACCTCCCTGATTACTATAAG ATCATTAAAACGCCATGGATATGGGAACA ATAAAGAAGCGCTTGGAAAACAACATTAC TGGAAATGCTCAGGAATGTATCCAGGACTTC AACACTATGTTACAAATTGTTACATCTAC AACAGCCTGGAGATGACATAGTCTTAATG GCAGAAGCTCTGGAAAAGCTCTTGCAA AAAATAATGAGCTACCCACAGAAGAATGA CAGTAAAGGTGGATACGGATCCGAA
Final protein sequence (tag sequence in lowercase): mhyyyyhssgvdlgtenlyfq^MNPPPET SNPNKPKRQTNQLQYLLRVVLKTLWKHQFA WPFQQPVDAVKLNLPDYYKIIKTPMDMGTI KKRLENNYYWNAQECIQDFNTMFTNCYIYN KPGDDIVLMAEALEKLFQKINELPTEE ^ TEV cleave site
Tags and additions: Cleavable N-terminal His6 tag.
Host: BL21 (DE3)R3-pRARE2 (Phage resistant strain)
Growth medium, induction protocol: 10 ml from a 50 ml overnight culture containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol were used to inoculate each of two 1 liter cultures of TB containing 50 µg/ml kanamycin and 34 µg/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 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 30 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml 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 Buffers: 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 spec characterization: LC- ESI -MS TOF gave a measured mass of 15083 for construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 10.3 mg/ml using an Amicon 3kDa cut-off concentrator.

Crystallization: Crystals were grown at 4°C in 300 nl sitting drops from a 1:1 ratio of protein (10.1 mg/ml + 2 mM ligand JQ1/SGCBD01) to reservoir solution containing 0.2 M NaI, 0.1 M BTProp pH 8.5, 20% PEG3350 and 10% ethylene glycol.

Mass spectrometry characterization: LC- ESI -MS TOF gave a measured mass of 22966 for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 10 mg/ml using an Amicon 10 kDa cut-off concentrator.

Crystallisation: Crystals were grown at 20°C in 300 nl sitting drops from a 1:1 ratio of protein to reservoir solution containing 20% PEG-smear (PEG3350 & PEG MME5K), 0.1 M HEPES pH 7.5

Data Collection: Crystals were cryo-protected using the well solution supplemented by 25% ethylene glycol and flash frozen in liquid nitrogen.

X-ray source: Diffraction data were collected from a single crystal on a Rigaku FRE with an RAXIS IV detector at a single wavelength of 1.542 Å and the structure was refined to 1.6 Å.

Phasing: The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.