

Materials and Method

Note: To our best knowledge, this should represent an accurate description of the materials and methods required to reproduce our work. If any of the content on this page is difficult to interpret or should you have trouble repeating our work, do not hesitate to [contact us](#) as soon as possible in order for us to provide additional information and advice.

Entry Clone Source: Synthetic
Entry Clone Accession: n/a
SGC Construct ID: PHIPA-c034
GenBank GI number: gi 34996489
Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]
Amplified construct sequence: CATATGCACCATCATCATCATCATTCTTC TGGTGTAGATCTGGGTACCGAGAACCTGT ACTTCCAATCCATGGATCATCAGCCGCGT CGTCGTCTGCGCAACCGTGCGCAGAGCTA TGATATCCAGGCGTGGAACAAACAGTGCG AAGAACTGCTGAATCTGATTTTTCAGTGT GAAGATAGCGAACCGTTTCGTCAGCCGCT TGATCTGCTGGAATATCCGGATTATCGCG ATATCATTGATACCCCGATGGATTTTGCG ACCGTGCGCGAAACCTGGAAGCGGGTAA CTATGAAAGCCCGATGGAAGTGCAAAAG ATGTGCGCCTGATTTTATGCAACAGCAAA GCCTATACCCCGAGCAAACGTAGCCGTAT CTATAGCATGAGCCTGCGTCTGAGCGCGT TTTTTGAAGAACACATTAGCAGCGTGCTG AGCGATTATAAAAGCGCGCTGCGCTTTCA TAAACGTTGACAGTAAAGGTGGATACGGA TCCGAA
Tags and additions: Cleavable N-terminal His6 tag.
Final protein sequence: MHHHHHHSSGVDLGTENLYFQ^SMDHQPR RRLRNRAQSYDIQAWKKQCEELNLIFQC EDSEPFRRQVDLLEYPDYRDIIDTPMDFA TVRETLEAGNYESPMELCKDVRLIFSNSK AYTPSKRSRIYSMSLRLSAFFEEHISSVL SDYKSALRFHKKR ^ TEV cleave site
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.

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).
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.
Buffers: 10 mM HEPES, pH 7.5; 500 mM NaCl, 5% glycerol.
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 spec characterization: LC- ESI -MS TOF gave a measured mass of 1607 for this construct as predicted from the sequence of this protein.
Protein concentration: Protein was concentrated to 18.6 mg/ml using an Amicon 3 kDa cut-off concentrator.
Crystallization: Crystals were grown at 4°C in 300 nl sitting drops from a 1:2 ratio of protein to reservoir solution containing 11% PEG 3350, 4 % glycerol, 0.1 M acetate pH 4.6. 2mM NMP was added to the protein solution.
Data Collection: Crystals were cryo-protected using the well solution supplemented by 30% glycerol and flash frozen in liquid nitrogen. X-ray source: Diffraction data were collected from a single crystal on Diamond beamline I04 at a single wavelength of 0.9762 Å and the structure was refined to 2.25 Å. Phasing: The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.