

**Entry Clone Source:** Synthetic

**Entry Clone Accession:** GI:20357585

**Vector:** pNIC28-Bsa4 Details [[PDF](#)]; Sequence [ [FASTA](#) ] or [ [GenBank](#) ]

**Amplified DNA sequence:**

CATATGCACCATCATCATCATTC  
TTCTGGTGTAGATCTGGGTACCGAGA  
ACCTGTACTTCCAATCCATGAGCATT  
CATCGTCGTCGTACCGATCCGATGGT  
GACCCTGAGCAGCATTCTGGAAAGCA  
TTATCAACGATATGCGCGATCTGCCG  
AACACCTATCCGTTTCATACCCCGGT  
TAACGCGAAAAGTGGTTAAAGATTATT  
ATAAAATTATCACCCGTCCGATGGAT  
CTGCAGACCCTGCGCGAAAACGTTCTG  
TAAACGTCTGTATCCGAGCCGCGAAG  
AATTTTCGTGAACATCTGGAAGTATT  
GTGAAAAATAGCGCGACCTATAATGG  
CCCGAAACACAGCCTGACCCAGATTA  
GCCAGAGCATGCTGGATCTGTGCGAT  
GAAAAACTGAAAGAAAAAGAAGATAA  
ACTGGCGCGCCTGGAAAAAGCCATTA  
ATCCGCTGCTGGATGATGATGATCAG  
GTGGCCTTTAGCTTCATTCTGGATAA  
CATTGTGACCCAGAAAATGATGGCCG  
TGCCGGATAGCTGGCCGTTTCATCAC  
CCGGTGAACAAAAAATTTGTGCCGGA  
TTATTATAAAGTGATTGTTAACCCGA  
TGGATCTGGAAACCATTCGTAAAAAT  
ATTAGCAAACACAAATATCAGAGCCG  
CGAAAGCTTTCTGGATGATGTGAATC  
TGATTCTGGCCAATAGCGTTAAATAT  
AATGGTCCGGAAAGCCAGTATACCAA  
AACCGCGCAGGAAATTGTGAACGTTT  
GTTATCAGACCCTGACCGAATATGAT  
GAACATCTGACCCAGCTGGAAAAAGA  
TATCTGCACCGCCAAAGAAGCGGCGC  
TGGAAGAAGCGGAACTGGAAAGCCTG  
GATTGACAGTAAAGGTGGATACGGAT  
CCGAA

**Final protein sequence (Tag sequence in lowercase):**

mhhhhhssgvdlgtenlyfq^smQC  
mhhhhhssgvdlgtenlyfq^smSI  
HRRRTDPMVTLSSILESIINDMRDLP  
NTYPFHTPVNAKVVKDYKIIITRPM  
LQTLRENVKRKLYPSREEFRELLELI  
VKNSATYNGPKHSLTQISQSMLDLCD  
EKLKEKEDKLARLEKAINPLLDDDDQ  
VAFSFI LDNI VTQKMMAVPDSWPFHH  
PVNKKFVPDYKVI VNPMDLETIRKN  
ISKHKYQSRESFLDDVNLI LANSVKY  
NGPESQYTKTAQEIVNVCYQTLTEYD

EHLTQLEKDICTAKEAALEEEAELES

D

^ TEV cleavage 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<sub>600</sub> reached ~2.5 then the temperature was adjusted to 18°C.

Expression was induced overnight using 0.1 mM IPTG at an OD<sub>600</sub> 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. DNA was precipitated with 0.15% PEI. 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 washed first with 30 ml of binding buffer then with 30 ml of 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 Buffers:**

**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.

**Protein concentration:** The protein was concentrated to 20 mg/ml using an Amicon 3 kDa cut-off concentrator.

**Mass spectrometry characterization:**

**Measured mass::** 30925.3 Da

LC-ESI-MS TOF gave a measured mass of 30925.3 Da for the TEV cleaved protein as predicted from the sequence of this protein.

**Crystallisation:** Protein buffer was exchanged to 10 mM HEPES pH7.5 and 300 mM NaCl. The protein was concentrated to 10 mg/ml using an Amicon 3 kDa cut-off concentrator. Crystals were grown at 4°C in 300 nl sitting drops from a 1:1 ratio of protein to reservoir solution containing 0.1M Tris.HCl pH 9.0, 1.1M (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>

**Data collection:****X-ray source:** Diamond I02**Resolution:** 0.97Å

Crystals were cryo-protected using the well solution supplemented by 2 M Li<sub>2</sub>SO<sub>4</sub> and flash frozen in liquid nitrogen. Data were collected at Diamond beamline I02 at a wavelength of 0.97Å.

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