

**Entry Clone Source:** MGC

**Entry Clone Accession:** gi|3504538

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

**Amplified DNA sequence:**

CATATGCACCATCATCATCATTC  
TTCTGGTGTAGATCTGGGTACCGAGA  
ACCTGTACTTCCAATCCATGACAGAT  
CAGGCCTTTGTGACACTAACCACAAA  
CGATGCCTACGCCAAAGGTGCCCTGG  
TCCTGGGATCATCTCTGAAACAGCAC  
AGGACCACCAGGAGGCTGGTCGTGCT  
CGCCACCCCTCAGGTCTCAGACTCCA  
TGAGAAAAGTTTTAGAGACAGTCTTT  
GATGAAGTCATCATGGTAGATGTCTT  
GGACAGTGGCGATTCTGCTCATCTAA  
CCTTAATGAAGAGGCCAGAGTTGGGT  
GTCACGCTGACAAAGCTCCACTGCTG  
GTCGCTTACACAGTATTCAAAATGTG  
TATTCATGGATGCAGATACTCTGGTC  
CTAGCAAATATTGATGATCTTTTTGA  
CAGAGAAGAATTGTCAGCAGCACCAG  
ACCCAGGGTGGCCTGACTGCTTCAAT  
TCCGGAGTCTTCGTTTATCAGCCTTC  
AGTTGAAACATACAATCAGCTGTTGC  
ATCTTGCTTCTGAGCAAGGTAGTTTT  
GATGGTGGGGACCAAGGCATACTGAA  
CACATTTTTTTAGCAGCTGGGCAACAA  
CAGATATCAGAAAACACCTGCCGTTT  
ATTTATAACCTAAGCAGCATCTCTAT  
ATACTCCTACCTCCCGGCATTTAAAG  
TGTTTGGTGCAAGTGCCAAAGTTGTG  
CATTTCTGTTGGGACGAGTCAAACCATG  
GAATTATACTTATGATCCCAAAACAA  
AAAGTGTCAAAAGTGAGGCCCATGAT  
CCCAACATGACTCATCCAGAGTTTCT  
CATCCTGTGGTGGAACATCTTTACCA  
CCAACGTTTTACCTCTGCTTCAATGA  
CAGTAAAGGTGGATACGGATCCGAA

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

mhhhhhhsgvdlgtenlyfq^smTD  
QAFVTLTTNDAYAKGALVLGSSLKQH  
RTTRRLVVLATPQVSDSMRKVLETVF  
DEVIMVDVLDSDSAHLTLMKRPELG  
VTLTKLHCWSLTQYSKCVFMDADTLV  
LANIDDLFDREELSAAPDPGWPDCFN  
SGVFVYQPSVETYNQLLHLASEQGSF  
DGGDQGILNTFFSSWATTDIRKHLPF  
IYNLSSISIIYSYLPFAKVFASAKVV  
HFLGRVKPWNYYTDPKTKSVKSEAHD  
PNMTHPEFLILWWNIFTTNVLP LLQ

^ TEV cleavage site

**Tags and additions:** Cleavable N-terminal His6 tag.

**Host:** BL21 (DE3)R3-pRARE2 (Phage resistant strain)

**Growth medium, induction protocol:** A glycerol stock was used to inoculate 50 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to inoculate 6L of TB media (7.5 ml starter culture used per 1L) containing 50 µg/ml kanamycin. When the OD<sub>600</sub> reached approximately 0.8 the temperature was reduced to 18°C and after a further 30 minutes the cells were induced by the addition of 0.1 mM IPTG. The expression was continued overnight.

**Binding buffer:** 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 10 mM Imidazole pH 7.4, 0.5 mM TCEP, 1 tablet per 50 ml protease inhibitor cocktail EDTA-free (Roche)

**Extraction buffer, extraction method:** Cell pellets were dissolved in approximately 150ml lysis buffer and broken by passing through a high pressure homogenizer at 15,000 psi for 4 cycles. The cell debris was pelleted at 35,000 x g and the supernatant used for further purification

**Column 1:** Ni-NTA (2.5 ml volume in a gravity-flow column).

**Column 1 Buffers:**

**Binding buffer:** 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 10 mM Imidazole pH 7.4, 0.5 mM TCEP

**Wash buffer:** 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.4, 0.5 mM TCEP

**Elution buffer:** Elution Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4, 0.5 mM TCEP

**Column 1 Procedure:** The clarified cell extract was incubated with 2.5 ml of Ni-NTA pre-equilibrated with lysis buffer for 1 hour at 4°C with rotation after which it was passed through a glass column. The column was then washed with Binding Buffer (50 ml) and Wash Buffer (50 ml). The protein was eluted with 30 ml of Elution Buffer in 6 ml fractions

**Column 2:** Superdex 75 16/60 Gel Filtration

**Column 2 Buffers:**

**GF Buffer:** 10 mM Hepes pH 7.4, 500 mM NaCl, 0.5 mM TCEP, 5% Glycerol.

**Column 2 Procedure:** The elution buffer fractions from column 1 were pooled and concentrated to 5 ml with a 10 kDa mwco spin concentrator and injected onto an s75 16/60 column (pre-equilibrated in GF Buffer) at 1.0 ml/min. 1 ml fractions were collected. The protein eluted at between 50 ml and 55 ml volume.

**Column 3:** Ni-NTA rebind

**Column 3 Procedure:** Protein fractions eluted at 50-55 ml from S75 gel filtration were pooled and incubated with 1:20 mol:mol TEV protease overnight at 4°C. Then protein plus TEV was passed through a column containing 0.5 ml Ni-NTA pre-equilibrated with GF Buffer. Column was washed 1ml of GF Buffer. Flow-through and wash were pooled

**Protein concentration:** 26.0 mg/ml using Millipore 10k mwco concentrators.

**Mass spectrometry characterization:**

**Measured mass:** 29568.1 Da

**Expected mass:** 29566.7 Da

**Crystallisation:** Prior to crystallization, protein was pre-incubated with 1mM MnCl<sub>2</sub> and 1mM uridine phosphate glucose (UDPG). Crystals were grown by vapour diffusion in sitting drop at 20°C. A sitting drop consisting of 50 nl protein and 100 nl well solution was equilibrated against well solution containing 0.2M lithium sulfate, 0.1M Tris pH 8.5 and 25% w/v

PEG3350. Crystals were mounted in the presence of 25% (v/v) ethylene glycol and flash-cooled in liquid nitrogen.

**Data collection:**

**X-ray source:** FRE superbright, single wavelength

**Resolution:** 2.0Å