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

**Amplified construct sequence:**
TACTTCCAATCCATGACCGTTGATCCGATT
GCCGTGTGTCATGAACTGTATAATACCATT
CGCGATTATAAAGATGAACAGGGTCGCCTG
CTGTGCGAACTGTTCATTCGCGCCCCGAAA
CGTCGCAATCAGCCGGATTATTATGAAGTG
GTTAGCCAGCCGATCGATCTGATGAAAATT
CAGCAGAAACTGAAAATGGAAGAATATGAT
GATGTGAACCTGCTGACCGCGGATTTTCAG
CTGCTGTTTAATAATGCGAAAAGCTATTAT
AAACCGGATAGCCCGGAATATAAAGCCGCG
TGCAAACTGTGGGATCTGTATCTGCGTACC
CGCAATGAATTTGTGCAGAAAGGTGAATGA
CAGTAAAGGTGGATA

**Final protein sequence (tag sequence in lowercase):**
mhhhhhhssgvdlgtenlyfq^sMTVDPIAVCHEL
YNTIRDYKDEQGRLLCELFIRAPKRRN
QPDYYEVVSQPIDLMKIQQKLKMEEY
DDVNLLTADFQLLFNNAKSYYKPDSP
EYKAACKLWDLYLRTRNEFVQKGE
^ 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 litre 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 OD600 of 3.0. The cells were collected by centrifugation and the pellet resuspended 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 16,500 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; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 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 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

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

**Column 2 Buffers**: 10 mM HEPES, pH 7.5; 500 mM NaCl, 5% glycerol

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| **Column 2 Procedure:** PB1 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. |
| **Column 3:**Ni-affinity. Ni-sepharose (Amersham), 2 ml of 50% slurry in a Bio-rad poly-prep column, washed with binding buffer. |
| **Column 3 Buffers: Binding buffer:** 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol; **Wash buffer:** 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM Imidazole |
| **Column 3 Procedure:** Gel filtration fractions containing the protein were pooled and loaded by gravity flow on the Ni-sepharose column. After loading a further 4 ml of binding buffer was added and the full flow through was collected. The column was then washed with 5 ml wash buffer at gravity flow. Finally, 6 ml of elution buffer was added. Flow through, wash and elution fractions were analysed by SDS PAGE. The TEV-cleaved protein was mainly found in the wash fraction. 10 mM DTT was added for overnight storage. |
| **Mass spectrometry characterization:**LC- ESI -MS TOF gave a measured mass of 13878.9 for this construct as predicted from the sequence of this protein. |
| **Protein concentration:** Protein samples were concentrated to 12.32 mg/ml using an Amicon 3 kDa cut-off concentrator. |
| **Crystallization:** Crystals were grown at 4 oC in 400 nl sitting drops from a 2:1 ratio of protein to reservoir solution containing 2.1 M Na(malate) pH 7.0. |
| **Data Collection:** Crystals were cryo-protected using the well solution supplemented with 40% PEG 400 and flash frozen in liquid nitrogen. **X-ray source:** Diffraction data were collected from a single crystal on a Rigaku FR-E SuperBright at a single wavelength of 1.5 Å and the structure was refined to 1.63 Å. **Phasing:** The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model. |