**Entry Clone Source:** Synthetic

**Entry Clone Accession:** n/a

**SGC Construct ID:** KIAA1240A-c007

**GenBank GI number:** gi|51460532

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

**Amplified construct sequence:**
CATATGCACCATCATCATCATCATTCTTCT
GGTGTAGATCTGGGTACCGAGAACCTGTAC
TTCCAATCCATGGAAGATCAGGAAGAAAAT
ACCCTGCGCGAACTGCGCCTGTTTCTGCGT
GATGTGACCAAACGTCTGGCGACCGATAAA
CGTTTTAATATTTTTAGCAAACCGGTGGAT
ATTGAAGAAGTGAGCGATTATCTGGAAGTG
ATTAAAGAACCGATGGATCTGAGCACCGTG
ATTACCAAAATCGATAAACATAATTATCTG
ACCGCGAAAGATTTCCTGAAAGATATTGAT
CTGATTTGCAGCAACGCGCTGGAATATAAC
CCGGATAAAGATCCGGGTGATAAAATTATT
CGTCATCGCGCGTGTACCCTGAAAGATACC
GCGCATGCGATTATCGCCGCCGAACTGGAT
CCGGAATTTAACAAACTGTGCGAAGAAATC
AAAGAAGCGCGTATTAAACGTGGCTGACAG
TAAAGGTGGATACGGATCCGAA

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

**Final protein sequence (tag sequence in lowercase):**
mhhhhhhssgvdlgtenlyfq^SMEDQEEN
TLRELRLFLRDVTKRLATDKRFNIFSKPVD
IEEVSDYLEVIKEPMDLSTVITKIDKHNYL
TAKDFLKDIDLICSNALEYNPDKDPGDKII
RHRACTLKDTAHAIIAAELDPEFNKLCEEI
KEARIKRG
^ 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.

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| **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:**<br>**Binding buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, 5% glycerol<br>**Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol<br>**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 Buffer:** 10 mM HEPES, pH 7.5; 500 mM NaCl, 5% glycerol |
| **Column 2 Procedure:** KIAA1240A 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. |
| **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 Buffer**: 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol |
| **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 buffer were added and the full flow through was collected. The column was then washed with 5 ml wash buffer at gravity flow. Flow through, and wash fractions were analyzed by SDS PAGE. The TEV-cleaved protein was mainly found in the flow-through fraction. |
| **Mass spectrometry characterization:** LC- ESI -MS TOF gave a measured mass of 15808 for this construct as predicted from the sequence of this protein. |
| **Protein Concentration:** Protein was concentrated to 14.1 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 20 % IPA, 0.1 M tris pH 8.5 |
| **Data Collection:** Crystals were cryo-protected using the well solution supplemented by 33 % glycerol and flash frozen in liquid nitrogen.<br>**X-ray source:** Diffraction data were collected from a single crystal on Diamond beamline I04 at a single wavelength of 0.9204 Å and the structure was refined to 2.34 Å.<br>**Phasing:** The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model. |