Entry Clone Source: Synthetic

Entry Clone Accession: n/a

SGC Construct ID: EP300A-c004

GenBank GI number: gi|50345997

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

Amplified construct sequence:
TACTTCCAATCCATGGCGCCGGGCCAGAG
CAAAAAAAAAATTTTCAAACCGGAAGAAC
TGCGTCAGGCCCTGATGCCGACCCTGGAA
GCCCTGTATCGTCAGGATCCGGAAAGCCT
GCCGTTTCGCCAGCCGGTGGATCCGCAGC
TGCTGGGTATCCCGGATTATTTTGATATT
GTTAAAAGCCCGATGGATCTGAGCACCAT
TAAACGTAAACTGGATACCGGCCAGTATC
AGGAACCGTGGCAGTATGTTGATGATATT
TGGCTGATGTTTAATAATGCGTGGCTGTA
TAATCGCAAAACCAGCCGCGTTTATAAAT
ATTGTAGCAAACTGAGCGAAGTGTTTGAA
CAGGAAATTGATCCGGTGATGCAGAGCCT
GGGCTGACAGTAAAGGTGGATA

Tags and additions: Cleavable N-terminal His6 tag.

Final protein sequence (tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfq^sMAPGQS
KKKIFKPEELRQALMPTLEALYRQDPESL
PFRQPVDPQLLGIPDYFDIVKSPMDLSTI
KRKLDTGQYQEPWQYVDDIWLMFNNAWLY
NRKTSRVYKYCSKLSEVFEQEIDPVMQSL
G
^ 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 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 OD_{600} 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.

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.

Buffers: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 10 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).

Procedure: Supernatant was loaded by gravity flow on the Ni-sepharose column. The column was then washed with 30 ml wash buffer by 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 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

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

Procedure: EP300 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.

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.

Procedure: Gel filtration fractions containing EP300 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 EP300 protein was mainly found in the flow through fraction.

Mass spec characterization: LC- ESI -MS TOF gave a measured mass of 14639 for this construct as predicted from the sequence of this protein.

Protein concentration: The protein was concentrated to 9.0 mg/ml using an Amicon 3 kDa cut-off concentrator.

Crystallization: Crystals were grown at 4°C in 600 nl sitting drops from a 1:1 ratio of protein to reservoir solution containing 30% w/v PEG 3350; 0.2M ammonium sulfate; 5.5pH Bis-Tris.

Data Collection: Crystals were cryo-protected using 2M Li2SO4 and flash frozen in liquid nitrogen; **X-ray source:** Diffraction data were collected from a single crystal on beamline X10SA of the Swiss Light Source at a single wavelength of 1.000 Å and the structure was refined to 2.33 Å; **Phasing:** The structure was solved by molecular replacement using an ensemble of known bromodomain structures as a starting model.