Molecular Biology

Entry Clone Accession: AAB68164.1
Entry Clone Source: Collaborator, Sean Froese (Zurich)
SGC Construct ID: MET12SCA-c002
Protein Region: M1-P302
Vector: pNIC28-Bsa4
Tag: N-6HIS;N-TEV
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MHHHHHHSSGVDLGTENLYFQSMSIRDLYHARASPFISLEFFPPKTELGTRNLMERMHR
MTALDPLFITVTWGAGGTTAEKTLTLASLAQQTLNIPVCMHLTCTNTEKAIIDDALDRCY
NAGIRNILALRGDPPIGEDWLDSQSNESPFKYAVDLVRYIKQSYGDKFCVGVAAYPEGHC
EGEAEGHEQDPLKDLVYLKEKVEAGADFVITQLFYDVEKFLTFEMLFRERISQDLPLFPG
LMPINSYLLFHRAAKLSHASIPPAILSRFPPEIQSDDNAVKSIGVDILIELIQEIYQRTSGRIKG
FHFYTLNLEKAIAQIVSQSP
Sequence after tag cleavage:
SMSIRDLYHARASPFISLEFFPPKTELGTRNLMERMHRMTALDPLFITVTWGAGGTTAEK
TLTLASLAQQTLNIPVCMHLTCTNTEKAIIDDALDRCYNAGIRNILALRGDPPIGEDWLDS
QSNESPFKYAVDLVRYIKQSYGDKFCVGVAAYPEGHCEGEAEGHEQDPLKDLVYLKEKV
EAGADFVITQLFYDVEKFLTFEMLFRERISQDLPLFPGLMPINSYLLFHRAAKLSHASIPPA
ILSRFPPEIQSDDNAVKSIGVDILIELIQEIYQRTSGRIKGFHFYTLNLEKAIAQIVSQSP
DNA Sequence:
CATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGTCCATCAGAGATTTATATCATGCGAGGGCTTCCCCTTTTATATCGTT
AGAATTCTTCCCTCCAAAGACTGAATTAGGGACGAGAAATTTGATGGAACGTATGCAT
CGTATGACTGCTTTAGATCCACTGTTTATCACGGTTACTTGGGGAGCAGGTGGTACTAC
TGCGGAAAAGACTCTGACATTAGCTTCCTTGGCACAGCAGACACTAAATATACCAGTT
TGTATGCATTTGACCTGTACAAACACAGAAAAAGCCATCATTGATGATGCGCTGGATA
GATGTTATAATGCAGGAATCAGGAATATTTTGGCTCTTCGAGGTGACCCACCTATTGGG
GAAGATTGGCTAGATTCTCAATCGAACGAATCACCTTTTAAATATGCGGTTGATTTAGT
TCGTTATATCAAGCAAAGCTACGGAGACAAGTTCTGCGTCGGTGTTGCAGCATATCCA
GAAGGTCATTGTGAAGGTGAAGCAGAAGGTCACGAGCAAGACCCATTGAAGGATTTG
GTATATTTAAAAGAAAAAGTTGAAGCTGGGGCCGATTTTGTGATAACACAACTGTTTT
ACGACGTTGAAAAATTCTTAACTTTTGAAATGCTATTTCGGGAACGGATTTCGCAAGA
TTTGCCCCTTTTCCCTGGGTTGATGCCTATTAACTCCTATCTGCTTTTCCACAGAGCAG
CAAAGTTATCACATGCATCTATTCCACCTGCAATACTGAGTAGGTTCCCCCCAGAAATC
CAATCGGATGATAATGCCGTGAAGTCCATTGGTGTGGACATTCTTATCGAATTGATTCA
GGAAATATATCAAAGAACATCTGGTAGAATTAAAGGGTTTCATTTCTATACATTAAATTT
GGAAAAGGCTATTGCTCAAATTGTCTCGCAATCTCCCTGACAGTAAAGGTGGATACGG
ATCCGAA

Protein Expression

Medium: TB
Procedure: The plasmid was transformed into E. coli BL21(DE3)-R3, cultured in 1 L Terrific Broth at 37°C to OD600 ~1.5, and induced with 0.5 mM IPTG overnight at 18oC. Harvested cells were homogenized in buffer A (50 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 20 mM imidazole) and centrifuged.

Protein Purification

Procedure: Proteins were purified by affinity (Ni-Sepharose; GE Healthcare) and size-exclusion (Superdex 200; GE Healthcare) chromatography. Protein was concentrated to 25 mg/mL and flash-cooled for storage at −80 °C.
Concentration: 25.0 mg/ml
Mass-spec Verification: Yes

Structure Determination

Crystallization:
Crystals were grown by siting drop vapour diffusion at 20°C, in mother liquor (0.2M Na/K tartrate, 20% PEG3350). Crystals were cryo-protected in mother liquor containing ethylene glycol (25% v/v) and flash-cooled in liquid nitrogen.

Protein Concentration: 25.0mg/ml
Data Collection: *Beamline:* Dmnd I04-1; *Resolution:* 1.56 Å
Data Processing: X-ray diffraction data were collected at the Diamond Light Source and processed using XIA2.