Molecular Biology

Entry Clone Accession: NM_182617 Variant
Entry Clone Source: Origene
SGC Construct ID: LOC148158A-c593
Protein Region: S32-Q577
Vector: pFB-LIC-Bse
Tag: N-6HIS;N-TEV
Host: Hi-Five

Sequence (with tag(s)):
MGHHHHHHSSGVDLGTENLYFQSMSLQWGHQEVPAKFNFASDVLDHWADMEKAGKRP
PSPALWWVNGKGKELMWNFRELSENSQQAANVLSGACGLQRGDRVAVVLPRVPEWWL
VILGCIRAGLIFMPGTIQMKSTDILYRLQMSKAKAIVAGDEVIQEVDTVASECPSLRIKLLV
SEKSCDGWLNFKKLLNEASTTHHCVETGSQEASAIYFTSGTSGLPKMAEHSYSSLGLKA
KMDAGWTGLQASDIMWTISDTGWILNILCSLMEPWALGACTFVHLLPKFDPLVILKTLSS
YPIKSMMGAPIVYRMLLQQDLSSYKFPHLQNCVTVGESLLPETLENWRAQTGLDIRESY
GQTETGLTCMVSKTMKIKPGYMGTAASCYDVQIIDDKGNVLPPGTEGDIGIRVKPIRPIGI
FSGYVDNPDKTAANIRGDFWLLGDRGIKDEDGYFQFMGRADDIINSSGYRIGPSEVENAL
MEHPAVVETAVISSPDPVRGEVVKAFVVLASQFLSHDPEQLTKELQQHVKSVTAPYKYPR
KIEFVLNLPKTVTGKIQRAKLRDKEWKMSGKARAQ
Sequence after tag cleavage:
SMSLQWGHQEVPAKFNFASDVLDHWADMEKAGKRPPSPALWWVNGKGKELMWNFRE
LSENSQQAANVLSGACGLQRGDRVAVVLPRVPEWWLVILGCIRAGLIFMPGTIQMKSTDI
LYRLQMSKAKAIVAGDEVIQEVDTVASECPSLRIKLLVSEKSCDGWLNFKKLLNEASTTH
HCVETGSQEASAIYFTSGTSGLPKMAEHSYSSLGLKAKMDAGWTGLQASDIMWTISDTG
WILNILCSLMEPWALGACTFVHLLPKFDPLVILKTLSSYPIKSMMGAPIVYRMLLQQDLSS
YKFPHLQNCVTVGESLLPETLENWRAQTGLDIRESYGQTETGLTCMVSKTMKIKPGYMG
TAASCYDVQIIDDKGNVLPPGTEGDIGIRVKPIRPIGIFSGYVDNPDKTAANIRGDFWLLGD
RGIKDEDGYFQFMGRADDIINSSGYRIGPSEVENALMEHPAVVETAVISSPDPVRGEVVKA
FVVLASQFLSHDPEQLTKELQQHVKSVTAPYKYPRKIEFVLNLPKTVTGKIQRAKLRDKE
WKMSGKARAQ
DNA Sequence:
CCATGGGCCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTG
TACTTCCAATCCATGTCCCTGCAGTGGGGCCACCAGGAAGTGCCGGCCAAGTTTAACT
TTGCTAGTGATGTGTTGGATCACTGGGCTGACATGGAGAAGGCTGGCAAGCGACCCC
CAAGCCCAGCCCTGTGGTGGGTGAATGGGAAGGGGAAGGAATTAATGTGGAATTTCA
GAGAACTGAGTGAAAACAGCCAGCAGGCAGCCAACGTCCTCTCGGGAGCCTGTGGC
CTGCAGCGTGGGGATCGTGTGGCAGTGGTGCTGCCCCGAGTGCCTGAGTGGTGGCTG
GTGATCCTGGGCTGCATTCGAGCAGGTCTCATCTTTATGCCTGGAACCATCCAGATGA
AATCCACTGACATACTGTATAGGTTGCAGATGTCTAAGGCCAAGGCTATTGTTGCTGGG
GATGAAGTCATCCAAGAAGTGGACACAGTGGCATCTGAATGTCCTTCTCTGAGAATTA
AGCTACTGGTGTCTGAGAAAAGCTGTGATGGGTGGCTGAACTTCAAGAAACTACTAA
ATGAGGCATCCACCACTCATCACTGTGTGGAGACTGGAAGCCAGGAAGCATCTGCCA
TCTACTTCACTAGTGGGACCAGTGGTCTTCCCAAGATGGCAGAACATTCCTACTCGAG
CCTGGGCCTCAAGGCCAAGATGGATGCTGGTTGGACAGGCCTGCAAGCCTCTGATATA
ATGTGGACCATATCAGACACAGGTTGGATACTGAACATCTTGTGCTCACTTATGGAAC
CTTGGGCATTAGGAGCATGCACATTTGTTCATCTCTTGCCAAAGTTTGACCCACTGGTT
ATTCTAAAGACACTCTCCAGTTATCCAATCAAGAGTATGATGGGTGCCCCCATTGTTTA
CCGGATGTTGCTACAGCAGGATCTTTCCAGTTACAAGTTCCCCCATCTACAGAACTGC
GTCACTGTAGGGGAGTCCCTTCTTCCAGAAACTCTGGAGAACTGGAGGGCCCAGACA
GGACTGGACATCCGAGAATCCTATGGCCAGACAGAAACGGGATTGACTTGCATGGTTT
CCAAGACAATGAAAATCAAACCAGGATACATGGGAACGGCTGCTTCCTGTTATGATGT

ACAGATCATAGATGATAAGGGCAACGTCCTGCCCCCCGGCACAGAAGGAGACATTGG
CATCAGGGTCAAACCCATCAGGCCTATAGGCATCTTCTCTGGCTATGTGGACAATCCCG
ACAAGACAGCAGCCAACATTCGAGGAGACTTTTGGCTCCTTGGAGACCGGGGAATCA
AAGATGAAGATGGGTATTTCCAGTTTATGGGACGGGCAGATGATATCATTAACTCCAGC
GGGTACCGGATTGGACCCTCGGAGGTAGAGAATGCACTGATGGAGCACCCTGCTGTG
GTTGAGACGGCTGTGATCAGCAGCCCAGACCCCGTCCGAGGAGAGGTGGTGAAGGC
ATTTGTGGTCCTGGCCTCGCAGTTCCTGTCCCATGACCCAGAACAGCTCACCAAGGAG
CTGCAGCAGCATGTGAAGTCAGTGACAGCCCCATACAAGTACCCAAGAAAGATAGAG
TTTGTCTTGAACCTGCCCAAGACTGTCACAGGGAAAATTCAACGAGCCAAGCTTCGA
GACAAGGAGTGGAAGATGTCCGGAAAAGCCCGTGCGCAGTGACAGTAAAGGTGGAT
ACGGATCCGAATTCGAGCTCCGTCGACAAGCTT

Protein Expression

Procedure: High five cells were grown in Sf900II medium supplemented with 1% FCS at 27°C. Cells were infected at a density of 2x10 ^{6} /ml with recombinant baculovirus (virus stock P2; 1ml of virus stock/1l of cell culture). 72 hours post-infection the cultures were collected and centrifuged for 30min at 2000rpm. The cell pellet was resuspended in cold PBS and centrifugation was repeated. The pellet was resuspended in lysis buffer (50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml)) and frozen at -80°C until purification.

Protein Purification

Procedure: The cell extract was incubated with Ni-Sepharose (0.250µl of resin/lysate from 1l of culture) during 1h at 4°C with gentle rotation. The resin was centrifuged at 1000g for 5min at 4°C, and washed 4x with 50ml of washing buffer. Resin was loaded on the gravity column and protein was eluted in 4 elution fractions (5ml each). Protein fractions were analysed by SDS-PAGE. Target protein containing fractions were concentrated using Amicon Ultra-15 concentrators with 10kDa cut-off, and purified on a gel filtration column (Superdex 200) on an Akta Purifier System. LOC148158 containing fractions were diluted with IEX buffer A to a concentration of NaCl of 50 mM. The protein was loaded onto a HiTrap Q Sepharose column, and eluted with a NaCl gradient (50-300mM). Fractions containing protein were analysed by SDS-PAGE.

Buffers:
Start buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP
Washing buffer: 50mM HEPES pH 7.5, 500mM NaCl, 10mM Imidazole, 5% glycerol, 1mM PMSF, 0.5mM TCEP
Elution buffer: 50mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 250mM Imidazole, 0.5mM TCEP
GF buffer: 10mM HEPES pH 7.5, 500mM NaCl, 5% glycerol, 0.5mM TCEP
IEX buffers: Buffer A: 50mM Tris pH 9.0; Buffer B: 50mM Tris pH 9.0, 1M NaCl

Concentration: 20.5 mg/ml
Mass-spec Verification: Yes

Structure Determination

Crystallization: Crystals were grown by vapor diffusion at 20°C in 150nl sitting drops. Ligand was added to a final concentration of 1mM prior to crystallisation. The drops were prepared by mixing 100nl of protein solution and 50nl of precipitant consisting of 0.1M Mg-formate and 15% PEG 3350. Crystals were flash-cooled in liquid nitrogen with 25 % glycerol as cryoprotectant.

Protein Concentration: 20.0mg/ml*;*
Data Collection: *Beamline:* FRER; *Resolution:* 2.4 Å