

Molecular Biology

Entry Clone Accession: IMAGE:4132071

Entry Clone Source: MGC

SGC Construct ID: FDP SA-c003

Protein Region: M67-K419

Vector: p11

Tag: N-6HIS;N-TEV

Host: BL21

Sequence (with tag(s)):

MGSSHHHHHHSSGRENLYFQGHMMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMG
HPEIGDAIARLKEVLEYNAIGGKYNRGLTVVVAFRELVPRKQDADSLQRAWTVGWCVE
LLQAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPY
YLNLIELFLQSSYQTEIGQTLDLLTAPQGNVDLVRFTKRYKSIVKYKTAFYSFYLP
IAAAMYMAGIDGEKEHANAKKILLEMGEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCS
WLTVQCLQRATPEQYQILKENYGGQKEAEKVARVKALYEELDLPVFLQYEEDSYSHI
MALIEQYAAPLPPAVFLGLARKIYKRRK

Sequence after tag cleavage:

GHMMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIG
GKYNRGLTVVVAFRELVPRKQDADSLQRAWTVGWCVELLQAFFLVADDIMDSSLTRRG
QICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYNLIELFLQSSYQTEIGQTL
DLTAPQGNVDLVRFTKRYKSIVKYKTAFYSFYLP
IAAAMYMAGIDGEKEHANAKKILLE
MGEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLTVQCLQRATPEQYQILKENYGGQ
KEAEKVARVKALYEELDLPVFLQYEEDSYSHI
MALIEQYAAPLPPAVFLGLARKIYKRRK

DNA Sequence:

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCAGAGAAAACCTGTATTTC
AGGGCCATATGATGAACGGAGACCAGAATTCAGATGTTTATGCCCAAGAAAAGCAGG
ATTTCGTTCACTTCTCCCAGATCGTTAGGGTGCTGACTGAGGATGAGATGGGGCA
CCCAGAGATAGGAGATGCTATTGCCCGGCTCAAGGAGGTCCTGGAGTACAATGCCATT
GGAGGCAAGTATAACCGGGGTTTGACGGTGGTAGTAGCATTCCGGGAGCTGGTGGAG
CCAAGGAAACAGGATGCTGATAGTCTCCAGCGGGCCTGGACTGTGGGCTGGTGTGTG
GAACTGCTGCAAGCTTCTTCCCTGGTGGCAGATGACATCATGGATTCATCCCTTACCCG
CCGGGGACAGATCTGCTGGTATCAGAAGCCGGGCGTGGGTTTGGATGCCATCAATGAT
GCTAACCTCCTGGAAGCATGTATCTACCGCCTGCTGAAGCTCTATTGCCGGGAGCAGC
CCTATTACCTGAACCTGATCGAGCTCTTCTGCAGAGTTCCTATCAGACTGAGATTGGG
CAGACCCTGGACCTCCTCACAGCCCCCAGGGCAATGTGGATCTTGTCTCAGATTCCTG
AAAAGAGGTACAAATCTATTGTCAAGTACAAGACAGCTTCTACTCCTTCTACCTTCT
ATAGCTGCAGCCATGTACATGGCAGGAATTGATGGCGAGAAGGAGCACGCCAATGCC
AAGAAGATCCTGCTGGAGATGGGGGAGTTCTTTCAGATTCAGGATGATTACCTTGACC
TCTTTGGGGACCCAGTGTGACCGGCAAAATTGGCACTGACATCCAGGACAACAAT
GCAGCTGGCTGGTGGTTCAGTGTCTGCAACGGGCCACTCCAGAACAGTACCAGATCC
TGAAGGAAAATTACGGGCAGAAGGAGGCTGAGAAAGTGGCCCGGGTGAAGGCGCTA
TATGAGGAGCTGGATCTGCCAGCAGTGTCTTGCAATATGAGGAAGACAGTTACAGCC
ACATTATGGCTCTCATTGAACAGTACGCAGCACCCCTGCCCCCAGCCGTCTTCTGGG
GCTTGCGCGCAAAATCTACAAGCGGAGAAAGTAGGGATCCTAA

Protein Expression

Procedure: Overnight cultures in TB (10 ml with 100 µg/ml ampicillin) were used to inoculate 1 litre of TB medium containing 100 µg/ml ampicillin. Cultures were grown at 37°C until they reached an OD₆₀₀ of 0.65 and then induced with 1 mM IPTG. The temperature was adjusted to

18°C and expression was allowed to continue overnight. The cells were collected by centrifugation, transferred to 50 ml tubes, resuspended in 25 ml binding buffer, and frozen at -80°C.

Protein Purification

Ni-affinity Ni-NTA (Qiagen), 4 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Binding buffer: 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, 5% glycerol. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol.

The supernatant was applied by gravity flow onto the Ni-NTA column. The column was sequentially washed with 30 ml binding buffer and 12.5 ml wash buffer. The protein was eluted by applying 12.5 ml of elution buffer and the eluate was collected in 1.5 ml fractions. The fractions were analyzed by SDS-PAGE gel, pooled and concentrated. FDPS was exchanged into crystallization buffer (10 mM Hepes, 0.5 M NaCl, 5% glycerol, pH 7.5) and concentrated to 13 mg/mL using a Millipore centrifugal concentrator with a 10 kDa MW cutoff.

Concentration: 13.0 mg/ml

Mass-spec Verification: Yes

Structure Determination

Crystallization: Crystals were grown at 20°C in 150 nl sitting drops by mixing 100 nl of protein solution and 50 nl of precipitant consisting of 40% PEG 300 and 0.1 M phosphate-citrate buffer pH 4.2.

Data Collection: *Beamline:* FRER; *Resolution:* 2.2 Å