Molecular Biology

Entry Clone Accession:
Entry Clone Source: Site-directed mutagenesis
SGC Construct ID: FDPSA-c022
Protein Region: M67-K419 (K200L point mutation)
Vector: p11
Tag: N-6HIS;N-TEV
Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):
MGSSHHHHHHSSGRENLYFQGHMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHP
EIGDAIARLKEVLEYNAIGGKYNRGLTVVVAFRELVEPRKQDADSLQRAWTVGWCVELL
QAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLN
LIELFLQSSYQTEIGQTLDLLTAPQGNVDLVRFTEKRYKSIVKYLTAFYSFYLPIAAAMYM
AGIDGEKEHANAKKILLEMGEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQCL
QRATPEQYQILKENYGQKEAEKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAP
LPPAVFLGLARKIYKRRK
Sequence after tag cleavage:
GHMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIGGK
YNRGLTVVVAFRELVEPRKQDADSLQRAWTVGWCVELLQAFFLVADDIMDSSLTRRGQI
CWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLNLIELFLQSSYQTEIGQTLDLL
TAPQGNVDLVRFTEKRYKSIVKYLTAFYSFYLPIAAAMYMAGIDGEKEHANAKKILLEM
GEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQCLQRATPEQYQILKENYGQKEA
EKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAPLPPAVFLGLARKIYKRRK
DNA Sequence:
ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCAGAGAAAACTTGTATTTCC
AGGGCCATATGATGAACGGAGACCAGAATTCAGATGTTTATGCCCAAGAAAAGCAGG
ATTTCGTTCAGCACTTCTCCCAGATCGTTAGGGTGCTGACTGAGGATGAGATGGGGCA
CCCAGAGATAGGAGATGCTATTGCCCGGCTCAAGGAGGTCCTGGAGTACAATGCCATT
GGAGGCAAGTATAACCGGGGTTTGACGGTGGTAGTAGCATTCCGGGAGCTGGTGGAG
CCAAGGAAACAGGATGCTGATAGTCTCCAGCGGGCCTGGACTGTGGGCTGGTGTGTG
GAACTGCTGCAAGCTTTCTTCCTGGTGGCAGATGACATCATGGATTCATCCCTTACCCG
CCGGGGACAGATCTGCTGGTATCAGAAGCCGGGCGTGGGTTTGGATGCCATCAATGAT
GCTAACCTCCTGGAAGCATGTATCTACCGCCTGCTGAAGCTCTATTGCCGGGAGCAGC
CCTATTACCTGAACCTGATCGAGCTCTTCCTGCAGAGTTCCTATCAGACTGAGATTGGG
CAGACCCTGGACCTCCTCACAGCCCCCCAGGGCAATGTGGATCTTGTCAGATTCACTG
AAAAGAGGTACAAATCTATTGTCAAGTACTTGACAGCTTTCTACTCCTTCTACCTTCCT
ATAGCTGCAGCCATGTACATGGCAGGAATTGATGGCGAGAAGGAGCACGCCAATGCC
AAGAAGATCCTGCTGGAGATGGGGGAGTTCTTTCAGATTCAGGATGATTACCTTGACC
TCTTTGGGGACCCCAGTGTGACCGGCAAAATTGGCACTGACATCCAGGACAACAAAT
GCAGCTGGCTGGTGGTTCAGTGTCTGCAACGGGCCACTCCAGAACAGTACCAGATCC
TGAAGGAAAATTACGGGCAGAAGGAGGCTGAGAAAGTGGCCCGGGTGAAGGCGCTA
TATGAGGAGCTGGATCTGCCAGCAGTGTTCTTGCAATATGAGGAAGACAGTTACAGCC
ACATTATGGCTCTCATTGAACAGTACGCAGCACCCCTGCCCCCAGCCGTCTTTCTGGG
GCTTGCGCGCAAAATCTACAAGCGGAGAAAGTAGGGATCCTAA

Protein Expression

Procedure: Overnight cultures in TB (10 ml with100 µg/ml ampicillin) were used to inoculate 1 litre of TB medium containing 100 µg/ml ampicillin. Cultures were grown at 37oC until they reached an OD600 of 0.65 and then induced with 1 mM IPTG. The temperature was adjusted to

18oC 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: 15.3 mg/ml
Mass-spec Verification: Yes

Structure Determination

Crystallization:
Crystallization Condition: TBA; 0.10M MgCl2; 20.0% PEG 6K; 10.0% EtGly*;*
Protein Concentration: 19.2mg/ml
Data Collection: *Beamline:* FREL; *Resolution:* 2.35 Å