

Molecular Biology

Entry Clone Accession:

Entry Clone Source: Site-directed mutagenesis

SGC Construct ID: FDPSA-c021 (Y204A point mutation)

Protein Region: M67-K419

Vector: p11

Tag: N-6HIS;N-TEV

Host: BL21(DE3)-R3-pRARE2

Sequence (with tag(s)):

MGSSHHHHHHSSGRENLYFQGHMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHP
EIGDAIARLKEVLEYNAIGGKYNRGLTVVAFRELVEPRKQDADSLQRAWTVGWCVELL
QAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLN
LIELFLQSSYQTEIGQTLDLLTAPQGNVDLVRFTEKRYKSIVKYKTAFASFYLPAAAAMYM
AGIDGEKEHANAKKILLEMGEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQCL
QRATPEQYQILKENYGGQKEAEKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAP
LPPAVFLGLARKIYKRRK

Sequence after tag cleavage:

GHMNGDQNSDVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIGGK
YNRGLTVVAFRELVEPRKQDADSLQRAWTVGWCVELLQAFFLVADDIMDSSLTRRGQI
CWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLNLIELFLQSSYQTEIGQTLDLL
TAPQGNVDLVRFTEKRYKSIVKYKTAFASFYLPAAAAMYMAGIDGEKEHANAKKILLEM
GEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVVQCLQRATPEQYQILKENYGGQKEA
EKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAPLPPAVFLGLARKIYKRRK

DNA Sequence:

ATGGGCAGCAGCCATCATCATCATCACAGCAGCGGCAGAGAAAACCTTGATTTCC
AGGGCCATATGATGCAGCACTTCTCCCAGATCGTTAGGGTGCTGACTGAGGATGAGAT
GGGGCACCCAGAGATAGGAGATGCTATTGCCCGGCTCAAGGAGGTCCTGGAGTACAA
TGCCATTGGAGGCAAGTATAACCGGGGTTTGACGGTGGTAGTAGCATTCCGGGAGCTG
GTGGAGCCAAGGAAACAGGATGCTGATAGTCTCCAGCGGGCCTGGACTGTGGGCTGG
TGTGTGGAAGTCTGCAAGCTTTCTTCCTGGTGGCAGATGACATCATGGATTCATCCCT
TACCCGCCGGGGACAGATCTGCTGGTATCAGAAGCCGGGCGTGGGTTCGGATGCCATC
AATGATGCTAACCTCCTGGAAGCATGTATCTACCGCCTGCTGAAGCTCTATTGCCGGGA
GCAGCCCTATTACCTGAACCTGATCGAGCTCTTCCTGCAGAGTTCCTATCAGACTGAG
ATTGGGCAGACCCTGGACCTCCTCACAGCCCCCAGGGCAATGTGGATCTTGTCAGAT
TCACTGAAAAGAGGTACAAATCTATTGTCAAGTACAAGACAGCTTTCGCCTCCTTCTA
CCTTCCTATAGCTGCAGCCATGTACATGGCAGGAATTGATGGCGAGAAGGAGCACGCC
AATGCCAAGAAGATCCTGCTGGAGATGGGGGAGTTCTTTCAGATTCAGGATGATTACC
TTGACCTCTTTGGGGACCCAGTGTGACCGGCAAAATTGGCACTGACATCCAGGACA
ACAAATGCAGCTGGCTGGTGGTTCAGTGTCTGCAACGGGCCACTCCAGAACAGTACC
AGATCCTGAAGGAAAATTACGGGCAGAAGGAGGCTGAGAAAGTGGCCCCGGGTGAAG
GCGCTATATGAGGAGCTGGATCTGCCAGCAGTGTTCTTGCAATATGAGGAAGACAGTT
ACAGCCACATTATGGCTCTCATTGAACAGTACGCAGCACCCCTGCCCCCAGCCGTCTT
TCTGGGGCTTGCGCGCAAAATCTACAAGCGGAGAAAGTAGGGATCCTAA

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: 12.74 mg/ml

Mass-spec Verification: Yes

Structure Determination

Crystallization:

Crystallization Condition: 0.01M ammonium₂SO₄ pH6.3; 14w/v PEG₆₀₀₀; 10v/v ethylene glycol

Protein Concentration: 15.5mg/ml; MgCl₂, Risedronate and Isopentenyl Pyrophosphate were added to protein prior to crystallization

Data Collection: *Beamline:* FRER; *Resolution:* 1.97 Å