

Molecular Biology

Entry Clone Accession: Codon-optimized

Entry Clone Source: Synthetic

SGC Construct ID: GMDSA-c026

Protein Region: R23-A372 (E157Q variant)

Vector: pLIC-SGC1

Tag: N-6HIS;N-TEV

Host: BL21 Gold

Sequence (with tag(s)):

MHHHHHHSSGVDLG TENLYFQSMRNVALITGITGQDGSYLA EFLLEKGYEVHGIVRRSSS
FNTGRIEHL YKNPQAHIEGNMKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDL
AEYTADV DGVGTLRL L DAVKTCGLINSVKFYQASTS QLYGKVQEIPQKETTPFYPRSPYG
AAKLYAYWIVVNFREAYNLFAVNGILFNHESPRRGANFVTRKISR SVAKIYLGQLECFSLG
NLDAKRDWGHAKDYVEAMWLMLQNDEPEDFVIATGEVHSVREFVEKSFLHIGKTIVWE
GKNENEVGRCKETGKVHVTVDLKY YRPTEVD FLQGDCTKAKQKLNWKPRVAFDELVR
EMVHADVELMRTNPNA

Sequence after tag cleavage:

SMRNVALITGITGQDGSYLA EFLLEKGYEVHGIVRRSSSFNTGRIEHL YKNPQAHIEGNM
KLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYTADV DGVGTLRL L DAVK
TCGLINSVKFYQASTS QLYGKVQEIPQKETTPFYPRSPYGA A KLYAYWIVVNFREAYNLFA
VNGILFNHESPRRGANFVTRKISR SVAKIYLGQLECFSLGNLDAKRDWGHAKDYVEAM
WLMLQNDEPEDFVIATGEVHSVREFVEKSFLHIGKTIVWEGKNENEVGRCKETGKVHVTV
VDLKY YRPTEVD FLQGDCTKAKQKLNWKPRVAFDELVRE MVHADVELMRTNPNA

DNA Sequence:

ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTACTT
CCAATCCATGCGTAACGTGGCGCTGATTACCGGCATTACCGGCCAGGATGGCAGCTAT
CTGGCGGAATTTCTGCTGGAAAAAGGCTATGAAGTGCATGGCATTGTGCGTCGTAGCA
GCAGCTTTAACACCGGCCGTATTGAACATCTGTATAAAAACCCGCAGGCGCATATTGA
AGGCAACATGAAACTGCATTATGGCGATCTGACCGATAGCACCTGCCTGGTGAAAATT
ATCAACGAAGTGAAACCGACCGAAATTTATAACCTGGGCGCGCAGAGCCATGTGAAA
ATTAGCTTTGATCTGGCGGAATATACCGCGGATGTGGATGGCGTGGGCACCCTGCGTC
TGCTGGATGCGGTGAAAACCTGCGGCCTGATTAAACAGCGTGAAATTTTATCAGGCGAG
CACCAGC_{caa}CTGTATGGCAAAGTGCAGGAAATTCGCGAGAAAGAAACCAACCCGTTT
TATCCGCGTAGCCCGTATGGCGCGGCCAAACTGTATGCGTATTGGATTGTGGTGAACCT
TCGTGAAGCGTATAACCTGTTTGCGGTGAACGGCATTCTGTTTAACCATGAAAGCCCG
CGTCGTGGCGCGAACTTTGTGACCCGTAAAATTAGCCGTAGCGTGGCGAAAATTTATC
TGGGCCAGCTGGAATGCTTTAGCCTGGGCAACCTGGATGCGAAACGTGATTGGGGCC
ATGCGAAAGATTATGTGGAAGCGATGTGGCTGATGCTGCAGAACGATGAACCGGAAG
ATTTTGTGATTGCGACCGGCGAAGTGCATAGCGTGC GTGAATTTGTGGAAGAGCTT
TCTGCATATTGGCAAACCATTTGTGTGGGAAGGCCAAAACGAAAACGAAGTGGGCCG
TTGCAAAGAAACCGGCAAAGTGCATGTGACCGTGGATCTGAAATATTATCGTCCGACC
GAAGTGGATTTTCTGCAGGGCGATTGCACCAAAGCGAAACAGAACTGAACTGGAA
ACCGCGTGTGGCGTTTGATGAACTGGTGCGTGAAATGGTGCATGCGGATGTGGAAC
TATGCGTACCAACCCGAACGCGTGA

Protein Expression

Procedure: GMDS was expressed in *E. coli* (BL21 Gold Magic) in Terrific Broth (TB) in the presence of 50 µg/ml of carbenicillin and kanamycin at 37°C to an OD₆₀₀ of 0.8. Cells were then

induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.5 mM, and incubated overnight at 15°C.

Protein Purification

Cultures were centrifuged and the cell pellets were resuspended in binding buffer (10 mM HEPES pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole) with protease inhibitor (0.1 M benzamidine-HCl, 0.1 M phenylmethyl sulfonyl fluoride, PMSF) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication. Lysate was cleared by centrifugation and passed through DE52 from Whatman in 0.5 M NaCl.

The cleared lysate was loaded onto a Ni-NTA (nickel-nitrilotriacetic acid) column from Qiagen at 4°C. The column was washed with wash buffer (10 mM HEPES pH 7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole), and the protein was eluted with elution buffer (10 mM HEPES pH 7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole).

The purified protein was dialyzed overnight into crystallization buffer (10 mM HEPES pH 7.5, 0.5 M NaCl) at 4°C and concentrated using Amicon Ultra centrifugal filter devices (Millipore). The protein was further purified to homogeneity by gel filtration (HighLoad 16/60 Superdex 200, Amersham Biosciences) equilibrated with crystallization buffer (10 mM HEPES pH 7.5, 0.5 M NaCl).

Concentration: 15.0 mg/ml

Mass-spec Verification: Yes

Structure Determination

Crystallization: Purified protein was crystallized using the hanging drop vapor diffusion method. In the presence of 1 mM NADP and 5 mM GDP-Mannose, crystals grew when the protein (15 mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop equilibrated against a reservoir solution containing 20% PEG3350 -- 0.15 M DL-malic acid.

Data Collection: *Beamline:* Dmnd I04-1; *Resolution:* 1.46 Å