

Molecular Biology

Entry Clone Accession: Codon-optimized

Entry Clone Source: Synthetic

SGC Construct ID: GMDSA-c024

Protein Region: R23-A372

Vector: pLIC-SGC1

Tag: N-6HIS;N-TEV

Host: B834(DE3)

Sequence (with tag(s)):

MHHHHHHSSGVDLG TENLYFQSMRNVALITGITGQDGSYLA EFLLEKGYEVH GIVRRSSS
FNTGRIEHL YKNPQA HIEGNM KLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDL
AEYTADV DGVGTLRL LDAVKTC GLINSVKFYQASTSELYGKVQEIPQKETTPFYPRSPYG
AAKLYAYWIVVNFREAYNLFAVNGILFNHESPRRGANFVTRKISR SVAKIYLGQLECFSLG
NLDAKR DWGHAKDYVEAMWLM LQNDPEDFVIATGEVHSVREFVEKSFLHIGKTIVWE
GKNENEVGRCKETGKVHVTVDLKY YRPTEVD FLQGDCTKAKQKLNWKPRVAFDELVR
EMVHADVELMRTNPNA

Sequence after tag cleavage:

SMRNVALITGITGQDGSYLA EFLLEKGYEVH GIVRRSSSFNTGRIEHL YKNPQA HIEGNM
KLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYTADV DGVGTLRL LDAVK
TCGLINSVKFYQASTSELYGKVQEIPQKETTPFYPRSPYGA AAKLYAYWIVVNFREAYNLFA
VNGILFNHESPRRGANFVTRKISR SVAKIYLGQLECFSLGNLDAKR DWGHAKDYVEAM
WLM LQNDPEDFVIATGEVHSVREFVEKSFLHIGKTIVWEGKNENEVGRCKETGKVHVTV
VDLKY YRPTEVD FLQGDCTKAKQKLNWKPRVAFDELVRE MVHADVELMRTNPNA

DNA Sequence:

ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTACTT
CCAATCCATGCGTAACGTGGCGCTGATTACCGGCATTACCGGCCAGGATGGCAGCTAT
CTGGCGGAATTTCTGCTGGAAAAAGGCTATGAAGTGCATGGCATTGTGCGTCGTAGCA
GCAGCTTTAACACCGGCCGTATTGAACATCTGTATAAAAACCCGCAGGCGCATATTGA
AGGCAACATGAAACTGCATTATGGCGATCTGACCGATAGCACCTGCCTGGTGAAAATT
ATCAACGAAGTGAAACCGACCGAAATTTATAACCTGGGCGCGCAGAGCCATGTGAAA
ATTAGCTTTGATCTGGCGGAATATACCGCGGATGTGGATGGCGTGGGCACCCTGCGTC
TGCTGGATGCGGTGAAAACCTGCGGCCTGATTAAACAGCGTGAAATTTTATCAGGCGAG
CACCAGCGAACTGTATGGCAAAGTGCAGGAAATTCCGCAGAAAGAAACCACCCCGTT
TTATCCGCGTAGCCCGTATGGCGCGGCCAAACTGTATGCGTATTGGATTGTGGTGAAC
TTCGTGAAGCGTATAACCTGTTTGCGGTGAACGGCATTCTGTTTAACCATGAAAGCCC
GCGTCGTGGCGCGAACTTTGTGACCCGTAAAATTAGCCGTAGCGTGGCGAAAATTTAT
CTGGGCCAGCTGGAATGCTTTAGCCTGGGCAACCTGGATGCGAAACGTGATTGGGGC
CATGCGAAAGATTATGTGGAAGCGATGTGGCTGATGCTGCAGAACGATGAACCGGAA
GATTTTGTGATTGCGACCGGCGAAGTGCATAGCGTGCGTGAATTTGTGGAAGAGAGCT
TTCTGCATATTGGCAAAACCATTTGTGTGGGAAGGCCAAAACGAAAACGAAGTGGGCC
GTTGCAAAGAAACCGGCAAAGTGCATGTGACCGTGGATCTGAAATATTATCGTCCGAC
CGAAGTGGATTTTCTGCAGGGCGATTGCACCAAAGCGAAACAGAAACTGAACTGGA
AACCGCGTGTGGCGTTTGTATGAACTGGTGCCTGAAATGGTGCATGCGGATGTGGAAC
TGATGCGTACCAACCCGAACGCGTGACAGTAAAGGTGGATAC

Protein Expression

Procedure: GMDS was expressed in *E. coli* (BL21 Gold Magic) in Terrific Broth (TB) in the presence of 50 ug/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 NADPH and 10 mM GDP-4F-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 -- 0.2 M ammonium citrate dibasic.

Data Collection: *Beamline:* Dmnd I24; *Resolution:* 1.939 Å