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)):
MHHHHHHSSGVDLGTENLYFQSMRNVALITGITGQDGSYLAEFLLEKGYEVHGIVRRSSS
FNTGRIEHLYKNPQAHIEGNMKLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDL
AEYTADVDGVGTLRLLDAVKTCGLINSVKFYQASTSELYGKVQEIPQKETTPFYPRSPYG
AAKLYAYWIVVNFREAYNLFAVNGILFNHESPRRGANFVTRKISRSVAKIYLGQLECFSLG
NLDAKRDWGHAKDYVEAMWLMLQNDEPEDFVIATGEVHSVREFVEKSFLHIGKTIVWE
GKNENEVGRCKETGKVHVTVDLKYYRPTEVDFLQGDCTKAKQKLNWKPRVAFDELVR
EMVHADVELMRTNPNA
Sequence after tag cleavage:
SMRNVALITGITGQDGSYLAEFLLEKGYEVHGIVRRSSSFNTGRIEHLYKNPQAHIEGNM
KLHYGDLTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYTADVDGVGTLRLLDAVK
TCGLINSVKFYQASTSELYGKVQEIPQKETTPFYPRSPYGAAKLYAYWIVVNFREAYNLFA
VNGILFNHESPRRGANFVTRKISRSVAKIYLGQLECFSLGNLDAKRDWGHAKDYVEAM
WLMLQNDEPEDFVIATGEVHSVREFVEKSFLHIGKTIVWEGKNENEVGRCKETGKVHVT
VDLKYYRPTEVDFLQGDCTKAKQKLNWKPRVAFDELVREMVHADVELMRTNPNA
DNA Sequence:
ATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTACTT
CCAATCCATGCGTAACGTGGCGCTGATTACCGGCATTACCGGCCAGGATGGCAGCTAT
CTGGCGGAATTTCTGCTGGAAAAAGGCTATGAAGTGCATGGCATTGTGCGTCGTAGCA
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CATGCGAAAGATTATGTGGAAGCGATGTGGCTGATGCTGCAGAACGATGAACCGGAA
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CGAAGTGGATTTTCTGCAGGGCGATTGCACCAAAGCGAAACAGAAACTGAACTGGA
AACCGCGTGTGGCGTTTGATGAACTGGTGCGTGAAATGGTGCATGCGGATGTGGAAC
TGATGCGTACCAACCCGAACGCGTGACAGTAAAGGTGGATAC

Protein Expression

Procedure: GMDS was expressed in *E. coli* (BL21 Gold Magic) in Terrific Broth (TB) in the presence of 50 ?g/ml of carbenicillin and kanamycin at 37°C to an OD_{600} 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

Procedure: 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^{o}C. The column was washed with wash buffer (10mM HEPES pH7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole), and the protein was eluted with elution buffer (10mM HEPES pH7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole).

The purified protein was dialyzed overnight into crystallization buffer (10 mM. HEPES pH7.5, 0.5 M NaCl) at 4^{o}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 1mM NADP and 5mM GDP-Mannose, crystals grew when the protein (15mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop equilibrated against a reservoir solution containing 20% PEG6000 -- 0.1M bicine pH 9.0.

Data Collection: *Beamline:* Dmnd I03; *Resolution:* 1.76 Å