GMDS

PDB:1T2A

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:9087147
Entry Clone Source:synthetic, codon-optimized gene
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfqghm. C-terminal: gs
Host:E.coli BL21 (Gold Magic)

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghmRNVALITGITGQDGSYLAEFLLEKGYEVHGIVRRSSSFNTGRIEHLYKNPQAHIEGNMKLHYGD
LTDSTCLVKIINEVKPTEIYNLGAQSHVKISFDLAEYTADVDGVGTLRLLDAVKTCGLINSVKFYQASTSELYGKVQEIPQKETTPF
YPRSPYGAAKLYAYWIVVNFREAYNLFAVNGILFNHESPRRGANFVTRKISRSVAKIYLGQLECFSLGNLDAKRDWGHAKDYVEAMW
LMLQNDEPEDFVIATGEVHSVREFVEKSFLHIGKTIVWEGKNENEVGRCKETGKVHVTVDLKYYRPTEVDFLQGDCTKAKQKLNWKP
RVAFDELVREMVHADVELMRTNPNAgs

Vector:p11

Growth

Medium:TB
Antibiotics:
Procedure:GMDSA was expressed in E. coli (BL21 Gold Magic) in Terrific Broth (TB) in the presence of 50 ÃÂµg/mL of carbenicillin and kanamycin at 37 ÃÂºC 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 ÃÂºC.

Purification

Buffers
Wash buffer: 10mM HEPES pH7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole
Elution buffer: 10mM HEPES pH7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole
Crystallization buffer: 10 mM HEPES, pH 7.5, 0.5 M NaCl
Procedure
The cleared lysate was loaded onto a Ni-NTA (nickel-nitrilotriacetic acid) column from Qiagen at 4degC. The column was washed with wash buffer, and the protein was eluted with elution buffer.

The purified protein was dialyzed overnight into crystallization buffer at 4degC and concentrated using Amicon Ultra centrifugal filter devices (Millipore). The protein was further purified to homogeneity by gel filtration (HighLoad 16/60 Superdex 75, Amersham Biosciences) equilibrated with crystallization buffer.

Extraction

Buffers
Binding buffer: 10 mM HEPES pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole
Procedure
Cultures were centrifuged and the cell pellets were resuspended in binding buffer with protease inhibitor (0.1ÂµM benzamidine-HCl, 0.1ÂµM 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.
Concentration:15 mg/mL
Ligand
GDPNADPH**MassSpec:**
Crystallization: Purified GMD R23-A372 was crystallized using the hanging drop vapor diffusion method. In the presence of 10mM GDP and 2mM NADPH, 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 21% PEG 4000, 0.1 M Tris pH 8.5, 0.2 M sodium acetate, and 3% dioxane or 75mM n-octyl-B-D-glucoside.
NMR Spectroscopy:
Data Collection:
Data Processing: