GIMAP2

PDB:3P1J

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:MGC cDNA library: AU69-B1:BC013934.1
Entry Clone Source:MGC
SGC Clone Accession:HPC09G-F03
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:GIMAP2:S19-K226, Tag removed
Tag intact protein has +57.4 Da mass difference compared with expected value, but tag removed one is consistent. There could be a modification with tag on.
Sequence:

gSRSELRIILVGKTGTGKSAAGNSILRKQAFESKLGSQTLTKTCSKSQGSWGNREIVIIDTPDMFSWKDHCEALYKEVQRCYLLSAP
GPHVLLLVTQLGRYTSQDQQAAQRVKEIFGEDAMGHTIVLFTHKEDLNGGSLMDYMHDSDNKALSKLVAACGGRICAFNNRAEGSNQ
DDQVKELMDCIEDLLMEKNGDHYTNGLYSLIQRSK

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in *E. coli* by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol at 37 °C. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 °C and the culutre was induced with 1 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were mixed with 3 mL 50% flurry of Talon Cobalt beads and incubated at 4 degree on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were then washed with 50 mL binding buffer twice, and finally the elution buffer. The elutant was

collected and treated with TEV protease overnight. The protease and uncut protein were removed by flowing the solution through a Talon cobalt open column and the flow-through were further purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter (mwco. 10 kDa). The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

Extraction

Procedure
Frozen cells from 2L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using microfluidizer at 16,000 PSI.
Concentration:8.0 mg/mL
Ligand
MassSpec: Native protein, uncut expected 25285.51, measured 25342.92, delta = +57.41 (Carbamidomethylation?)
Cut version expected: 23149.5, measured 23150.4, correct.
Crystallization: Crystal used for structure deterination was grown in optimized Red Wings H5 condition.
Crystal was grown in 1.4M NaCitrate, 0.1 M HEPES pH 7.6 in 2uL:2uL hanging drop setup. Five times molarity of GDP were added into the protein stock solution before setting up crystallization.
Crystals grow to a mountable size within 3 days. Paratone used as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: