CENTG3

PDB:3IHW

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:SGC cDNA library: DNA 03-G1:CENTG3
Entry Clone Source: Codon Devices Synthesized
SGC Clone Accession:HPC099-G11
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:CENTG3:P126-K291
Tag not removed
Sequence:

mhhhhhhssgrenlyfqgPELKVGIVGNLSSGKSALVHRYLTGTYVQEESPEGGRFKKEIVVDGQSYLLLIRDEGGPPELQFAAWVD
AVVFVFSLEDEISFQTVYNYFLRLCSFRNASEVPMVLVGTQDAISAANPRVIDDSRARKLSTDLKRCTYYETCATYGLNVERVFQDV
AQKVVALRKK

Vector:pET28-mhl (GI:134105571)

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 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degC and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degC.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Talon beads and incubated at 4°reeC 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 washing buffer containing 30 mM and 75 mM Imidazole, and finally the elution buffer. The flow-trough was collected and 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. The purity of the preparation is tested by SDS-PAGE to be greater than 95%. TEV protease failed to cleave the tag from the protein.

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 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer at 15,000 PSI.
Concentration:29.0 mg/mL
Ligand
MassSpec: Native expected 20771.55, measured 20772.7 (2009.06.03 gel filtration sample)There is one sample meausred at 2009-06-02 gave 20848.6 (also recorded in ELN EXP-09-AA6265), which is a 76 plus, could be caused by BME modification.
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. Crystal used for structure determination was grown in 15.9% PEG3350, 0.2M KF, 0.1M Bis-Tris pH 6.0 in sitting drop setup. Cryoprotectant used paratone.Crystals grow to a mountable size within several days.
NMR Spectroscopy:
Data Collection:
Data Processing: