PLXNC1

PDB:3KUZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Codon Devices Synthesized: SGC cDNA library: DNA 20-D3:PLXNC1
SGC Clone Accession:HPC09R-D12
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:PLXNC1:T1198-K1305
Tag was not removed
Sequence:

mhhhhhhssgrenlyfqgTVALNVVFEKIPENESADVCRNISVNVLDCDTIGQAKEKIFQAFLSKNGSPYGLQLNEIGLELQMGTRQ
KELLDIDSSSVILEDGITKLNTIGHYEISNGSTIKVFKK

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 16,000 rpm for 1 hour and the supernatants were mixed with 8 mL 50% flurry of Ni-NTA beads and incubated at 4 degC on rotary shaker for 1 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 5 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%.
During purification, the tag was not removed.

Extraction

Procedure
Frozen cells from 4L TB culture were thawed and resuspended in 500 mL extraction buffer with freshly added 0.5% CHAPS, and supplemented with 1.7 &milliL protease inhibitor cocktail (SIGMA Catalog # P8849), and 10 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using sonication at 120W for 8minutes on 50% duty cycle.
Concentration:7.0 mg/mL
Ligand
MassSpec: Native expected 14080.89, measured 14081.6
Crystallization: Crystallization was setup using in situ proteolysis method in sitting drops with Red Wings and SGC-I screens initially. Diffracting crystals were found from initial screen drops. Crystal used for structure determination was grown in 2.0 M (NH4)2SO4, 0.2 M NaCl, 0.1 M HEPES buffer pH 7.5, with 1:100 Chymotrypsin (w/w) in sitting drop setup.
Crystals grow to a mountable size after 3 days.
1.8 M Li2SO4 was used as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: