PLXND1

PDB:3H6N

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:CDV PLXND1 (SGC template 16-E4; camR)
Entry Clone Source: Codon Devices Synthesized
SGC Clone Accession:HPC078-G04
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude: Tag removed

PLXND1:A1553-L1678
Sequence:

gAKPRNLNVSFQGCGMDSLSVRAMDTDTLTQVKEKILEAFCKNVPYSQWPRAEDVDLEWFASSTQSYILRDLDDTSVVEDGRKKLNT
LAHYKIPEGASLAMSLIDKKDNTLGRVKDLDTEKYFHLVL

Vector:pET28-mhl

Growth

Medium:M9 Medium, SeMet
Antibiotics: Kanamycin 50 µg/mL Chloramphenicol 25 µg/mL
Procedure:LEX bubbling. The target protein was expressed in E.coli using prepacked M9 SeMet growth media kit (Medicilon) following manufacturer\'s instruction. Harvested cells were 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 Ni-NTA beads and incubated at 4 degC 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 were collected and digested with TEV protease. TEV protease was removed from the treated protein sample by adding 100 µL 50% flurry of Ni-NTA beads and the sample was purified with supderdex-75 gel filtration again.

Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter (m.w. cut-off 5,000). The purity of the preparation is tested by SDS-PAGE to be around 99%.

Extraction

Procedure
Frozen cells from 4L M9 medium were thawed and resuspended in 150 mL Binding Buffer with freshly added 0.5% CHAPS and 2mM BME, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 μL benzonase (Sigma Catalog # E1014, 250U/μL), and lysed using sonication.
Concentration:41.4 mg/mL
Ligand
MassSpec: SeMet: Expected 14498.0 (3 Met, tag cut), measured: 14498.0 (5%), 14574.62(76.62+, ~50%), 14650.6(152.60+, ~40%). The extra mass should come from BME modification.
Crystallization: Crystal used for phasing and refinement was grown using sitting drop vaporization method. The well solution contains 1.39 M NaCitrate, 0.1M Sodium Cocadylate buffer pH 5.2. The protein stock solution was adjusted to contain 15 mM TCEP, supplemented with 1:100 (w/w) Chymotrypsin, and 0.5uL protein solution was mixed with 0.5uL well solution using Mosquito instrument. The plate was stored at room temperature. Crystals appear in the drop in 3 to 5 days. Cryoprotectant used paratone.
NMR Spectroscopy:
Data Collection:
Data Processing: