PLXNB1

PDB:2R2O

Revision

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Revision Date:created
Entry Clone Accession: Provided by Dr. M.Buck (Case Western Reserve University, Cleveland, Ohio)
Entry Clone Source: Dr. Buck
SGC Clone Accession:HPC060-C12 (This construct will not be distributed since the DNA template used is a gift from Dr. Buck)
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgDVEYRPLTLNALLAVGPGAGEAQGVPVKVLDCDTISQAKEKMLDQLYKGVPLTQRPDPRTLDVEWRSGV
AGHLILSDEDVTSEVQGLWRRLNTLQHYKVPDGATVALVPCLTKHVLRENQ

Vector:pET28-mhl

Growth

Medium: Terrific Broth
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 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and chloramphenicol at 37 °C. When OD600 was ~3.0, the temperature of the media was lowered to 15 °C and the culutre was induced with 1mM IPTG, and the cells were allowed to grow overnight before harvesting and flash frozen in liquid nitrogen and stored at -80 °C before use.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatant was mixed with 5 mL 50% Ni-NTA beads, and incubated at 4 °C for 1 hours. The beads were then washed using washing buffer and the proteins eluted using 8 mL elution buffer twice. The elutants were pooled and loaded onto supderdex-75 gel filtration column. Eluted fractions were pooled and

concentrated using amicon centrifugal filter (m.w. cut-off 10,000). The purity of the proteins was higher than 95% judged by SDS-PAGE.

Extraction

Procedure
Frozen cells were thawed and suspended in 150 mL the binding buffer and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 2 microL (Sigma Catalog # E1014, 250U/microL) benzonase, and lysed using sonicator at 100W for 5 minutes (duty cycle: 10s on, 5s off)
Concentration:26.8mg/mL, in 50mM Tris pH 7.5 150mMNaCl 5mMDTT/MgCl2 buffer
Ligand
N/A**MassSpec:**Measured 15417.85, expected 15417.47SeMet labeled: measured 15511.67, expected 15513.36
Crystallization:RG05 optimizaiton: NaCl 2.6M 0.1M Tris pH 8.0 18% EG50mM Tris pH 7.5 150 mM NaCl 5 mM DTT/MgCl2
NMR Spectroscopy:
Data Collection:
Data Processing: