# PLXNA4

**PDB:**4E74

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**SGC cDNA collection: 16-F5
**Entry Clone Source:**Codon Devices
**SGC Clone Accession:**HPC09R-E09
**Tag:**N-terminal His6-tag, removed before crystallization
**Host:**BL21-V2R-pRARE2

## Construct

**Prelude:**PLXNA4A:D1488-T1603
**Sequence:**

gDKLIRQQIDYKTLVLSCVSPDNANSPEVPVKILNCDTITQVKEKILDAIFKNVPCSHRPKAADMDLEWRQGSGARMILQDEDITTK
IENDWKRLNTLAHYQVPDGSVVALVSKQVT

**Vector:**pET28-MHL

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**LEX Bubbling. The target protein was expressed in E. coli by inoculating each 50 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 30 mg/mL chloramphenicol at 37 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degree 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 degree.

## Purification

**Procedure**
The clarified lysate was mixed with 5 mL of 50% slurry of Talon beads and incubated at 4°C on rotary shaker for 1 hours. The mixture was then centrifuged at ~1000 x g (2000 rpms) for 5 min and the supernatant discarded. The beads were washed with 50 mL Binding Buffer followed by 50 mL of Washing Buffer. The protein was eluted with 10 ml of Elution Buffer. The eluted protein was mixed with TEV protease in a 1:4 molar ratio (TEV to protein) and dialyzed in Dialysis buffer overnight (Dialysis cassette has a 3 kDa molecular weight cut off). The dialyzed protein was further purified on a home-packed 26/60 Superdex-75 gel filtration column that was

pre-equilibrated with Gel Filtration Buffer. The flow rate is 2 mL/min. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter (3 kDa molecular weight cut off). The purity of the protein preparation was greater than 95% as judged by SDS-PAGE.

## Extraction

**Procedure**
Frozen cells from 4L TB culture were thawed and re-suspended in 500 mL Extraction Buffer. Cells were lysed by 50 times ten second pulses sonication on ice at 100W with ten second break between each pulse. The lysate was clarified by centrifugation at ~38000 x g (16,000 rpms) for 1 hour.
**Concentration:**39.2 mg/mL
**Ligand**
**MassSpec:**cut version, expected is 13132.0 Da and the measured value was 13132.2 Da
**Crystallization:**Crystal used for structure refinement was grown in RedWing (RW) screen condition A03, i.e., 25% PEG3350, 0.2M (NH4)OAc, 0.1M HEPES, pH7.5 in sitting drop setup, using 0.5µL protein + 0.5µL well solution against 100 µL reservoir buffer at room temperature. Crystals grow to a mountable size within 7 days and was dehydrated for one day by adding glycerol to 20% in the well solution before crystals are mounted.
Cryoprotectant used mineral oil
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**