PLXNB1 + RND1

PDB:2REX

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:PLXNB1: Provided by Dr. M.Buck (Case Western Reserve University, Cleveland, Ohio)
RND1: AT22-F2
Entry Clone Source:PLXNB1: Dr. Buck
Rnd1: MGC
SGC Clone Accession:PLXNB1 HPC060-C12
RND1 HPC060-H10
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

PLXNB1:gDVEYRPLTLNALLAVGPGAGEAQGVPVKVLDCDTISQAKEKMLDQLYKGVPLTQRPDPRTLDVEWRSGVAGHLILSDED
VTSEVQGLWRRLNTLQHYKVPDGATVALVPCLTKHVLRENQ

RND1:gRAPQPVVARCKLVLVGDVQCGKTAMLQVLAKDCYPETYVPTVFENYTACLETE
EQRVELSLWDTSGSPYYDNVRPLCYSDSDAVLLCFDISRPETVDSALKKWRTEILDYCPST
RVLLIGCKTDLRTDLSTLMELSHQKQAPISYEQGCAIAKQLGAEIYLEGSAFTSEKSIHSIF
RTASMLCLNKPSPLPQKSPV
Vector:pET28-mhl

Growth

Medium: Terrific Broth
Antibiotics:
Procedure:LEX Bubbling. The target proteins were 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 25 µg/mL chloramphenicol at 37 °C. When OD600 reached ~3.0, the temperature of the media was lowered to 15 °C and the culutre was induced with 1mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C before use.

Purification

Procedure
The lysates were centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% Ni-NTA beads, and incubated at 4 °C for 1 hours. The supernants were then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 10 mL washing buffer once. The proteins bound to beads were eluted using 8 mL elution buffer twice. The elutants were pooled and added with TEV protease (15 µL TEV at 4 mg/mL for every 1 mg target protein) and dialysed against gel filtration buffer at 4 °C overnight. The protein solutions were mixed with 0.5 mL 50% Ni-NTA beads and passed through another gravity column to remove His-tagged TEV protease. The flow-through was collected and loaded onto Supderdex-75 gel filtration column. Eluted fractions were pooled and concentrated using amicon centrifugal filter (m.w. cut-off 10,000). For RND1 GTPase, 10 times concentration of GppNHp was added to the protein before concentrating. 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 extraction buffer and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 2 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using sonicator at 100W for 5 minutes (duty cycle: 10\" on, 5\" off)
Concentration:5.0 mg/mL PLXNB1, 8.3 mg/mL RND1, i.e. 1:1 morality ratio
Ligand
GNP**MassSpec:**Tag-removed Rnd1 21924.61 expected 21924.18
Tag-removed PLXNB1 13281.51, expected 13281.17

Crystallization:PLXNB1 and RND1 were mixed at a molarity ratio of 1:1 before setting up crystallization. Mother liquor using RW-E04 20.0% PEG3350, 0.2M CaCl2. Proteins were in 50 mM Tris, pH 7.5 150 mM NaCl 5 mM DTT, 5 mM MgCl2 buffer. Sitting drop vaporization was set up. Crystals usually appear in two or three days. Most of them appear as stacked plates. Crystals also grow for PLXNB1 and RND1 without tag cleavage.
NMR Spectroscopy:
Data Collection:
Data Processing: