ARL5:Q15-L176

PDB:2H17

Revision

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Entry Clone Accession:BC001254
Entry Clone Source:MGC AT3-C4
SGC Clone Accession:HPC015-C08, ARL5_40
Tag:mgsshhhhhhssglvpr*gs
Host:E. coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsQEHKVIIVGLDNAGKTTILYQFSMNEVVHTSPTIGSNVEEIVINNTRFLMWDIGGQESLRSSWNTYYT
NTEFVIVVVDSTDRERISVTREELYKMLAHEDLRKAGLLIFANKQDVKECMTVAEISQFLKLTSIKDHQWHIQACCALTGEGLCQGL
EWMMSRL

Vector:p28a-thrombin-lic

Growth

Medium: Terrific Broth
Antibiotics:
Procedure: We prepared the seeds by inoculating freshly transforming E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After growing overnight, all of the seeds were inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin at 37ºC and grown to an OD600 of 3-4. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20ºC in SGC LEX bubbling system.

Purification

Procedure
The supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 3 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4ºC. The Ni-NTA column was washed with 150 mL of the wash buffer (10mM Tris pH7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). Five molar equivalents of GDP, 5 mM TCEP and 5 mM MgCl were added to the purified protein before concentration. The protein was concentrated using an Amicon Ultra centrifugal filter to the final

volume of 1 mL and the concentration of 10 mg/mL. About 4.5 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure

Concentration:10 mg/mL
Ligand
GDP**MassSpec:**
Crystallization: Crystallization was setup using sitting drop and 1:1 protein:mother liquor ratio. The crystal used for structure determination was grown in 2.0M sodium formate, 0.1M BTP, pH 7.0 at temperature 291K.

Structure solved by W.M. Rabeh, M&M last updated by ytong at 20080819 according to data in PolyMorph and M&M of other ARL5 structures
NMR Spectroscopy:
Data Collection:
Data Processing: