ARL5

PDB:1Z6Y

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:6912244
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host:E. coli BL21 (DE3)

Construct

Prelude:
Sequence:

```
gsGILFTRIWRLFNHQEHKVIIVGLDNAGKTTILYQFSMNEVVHTSPTIGSNVEEIVINNTRFLMWDIGGQESLRSSWNTYYTNTEF
VIVVVDSTDRERISVTREELYKMLAHEDLRKAGLLIFANKQDVKECMTVAEISQFLKLTSIKDHQWHIQACCALTGEGLCQGLEWMM
SRLKIR
```

Vector:p28a-LIC

Growth

Medium:
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 3.62. 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). The His tag was cleaved overnight at 4ºC using 1 unit of thrombin (Sigma T9681) per milligram of protein by dialyzing the sample overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5%

glycerol. The protein concentration was estimated based on the extinction coefficient of the protein, 33810 at 280 nm. 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 27.5 mg/mL. About 50 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure

Concentration:27 mg/mL
Ligand
MassSpec:
Crystallization: Purified ARL5 was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in one day when the protein (10 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio and the drop was equilibrated against a reservoir solution containing 25% PEG 4000, 0.1 M sodium acetate, pH 4.6, 0.2 M ammonium sulfate, 5% v/v 2-methyl-2,4-pentanediol. The crystals were flash frozen with the mother liquor with 27.5% PEG 4000 and 17.5% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: