

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: MGSSHHHHHSSGLVPR*GS

Host:E. coli BL21 (DE3)

Construct

Prelude:

Sequence:

gsGILFTRIWRFLFNHQEHKVIIVGLDNAGKTTILYQFSMNEVVHTSPTIGSNVEEIVINNTRFLMWDIGGQESLRSSWNTYYTNTFE
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 37°C 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 20°C 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 4°C. 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 4°C 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: