ARF4

PDB:1Z6X

Revision

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Entry Clone Accession:AT1-C03
Entry Clone Source:MGC
SGC Clone Accession:HPC003-A02
Tag:mgsshhhhhhssglvpr*gs
Host:E. coli BL21 (DE3)

Construct

Prelude:
Sequence:

gsGLTISSLFSRLFGKKQMRILMVGLDAAGKTTILYKLKLGEIVTTIPTIGFNVETVEYKNICFTVWDVGGQDRIRPLWKHYFQNTQ
GLIFVVDSNDRERIQEVADELQKMLLVDELRDAVLLLFANKQDLPNAMAISEMTDKLGLQSLRNRTWYVQATCATQGTGLYEGLDWL
SNELSKR

Vector:p28a-thrombin-lic

Growth

Medium: We prepared the seeds by inoculating freshly transforming E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After overnight growth, 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 2.28. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20ºC in the SGC LEX bubbling system.
Antibiotics:
Procedure:

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 pH7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The eluate was dialyzed overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol. The protein was treated with human alpha-thrombin, (Haematologic Technologies Inc.) about 10

units per mg protein, overnight at 4C and then the truncated protein was loaded onto 5 mL Ni-NTA column. The flowthrough containing the pure truncated protein was collected. The protein concentration was estimated based on the extinction coefficient of the protein, 29280 at 280 nm. Five molar equivalents of GDP, 5 mM TCEP and 5 mM MgCl2 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 15.0 mg/mL. About 27 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were suspended in 100 mL of the binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole) with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication. The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was used for subsequent steps of purification.
Concentration:15 mg/mL
Ligand
MassSpec:
Crystallization: Purified ARF4 was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in one week when the protein (15 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 1.4 M sodium citrate, 0.1 M Hepes, pH 7.5, 3% w/v 6-aminocaproic acid. The crystals were flash frozen with the mother liquor with 15% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: