ARFGAP1

PDB:3O47

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:AT50-C10 (ArfGAP1) and AT36-H1 (Arf1)
Entry Clone Source:MGC
SGC Clone Accession:HPC09V-F01
Tag:N-terminal: mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgMASPRTRKVLKEVRVQDENNVCFECGAFNPQWVSVTYGIWICLECSGRHRGLGVHLSFVRSVTMDKWKD
IELEKMKAGGNAKFREFLESQEDYDPCWSLQEKYNSRAAALFRDKVVALAEGREWSLESSPAQNWTPPQPRGLFGKKEMRILMVGLD
AAGKTTILYKLKLGEIVTTIPTIGFNVETVEYKNISFTVWDVGGQDKIRPLWRHYFQNTQGLIFVVDSNDRERVNEAREELMRMLAE
DELRDAVLLVFANKQDLPNAMNAAEITDKLGLHSLRHRNWYIQATCATSGDGLYEGLDWLSNQLRNQK

Vector:pET28-mhl (GI:134105571)

Growth

Medium: Terrific Broth medium in the presence of 50 ug/mL kanamycin and 25 ug/mL chloramphenicol
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in *E. coli* by inoculating 50 mL of overnight culture grown in Luria-Bertani medium into a 2 L of growth medium at 37 °C. When OD_{600} reached ~3.0, the temperature of the medium was lowered to 15 °C and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
The lysate was centrigued at 16,000 rpm for 1 hour and the supernatants were mixed with 12 mL 50% flurry of Ni-NTA beads and incubated at 4 oC on rotary shaker for 40 minutes. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were then pooled and washed with 50 mL washing buffer containing 10 mM and 30 mM Imidazole, and finally the elution buffer. The flow-trough was collected, added TEV protease (6ul per 1 mg

Protein). The mixture is dialyzed with the dialysis buffer for overnight. The TEV was removed by Ni-NTA beads and the protein sample was further purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter. The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

During purification, the tag was removed.

Extraction

Procedure
Frozen cells from 4L TB culture were thawed and resuspended in 500 mL extraction buffer with freshly added 0.5% CHAPS, and 1 mM PMSF/Benzomidine, and 12 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using sonication at 120W for 6'(duty cycle: 5" on, 5" off).
Concentration:22.0 mg/mL
Ligand
MassSpec: Native expected 35755.8, measured 35760.05
Crystallization: His-tag removed proteins were setup for crystallization in sitting drops using Red Wings and SGC-I screens initially. Diffracting crystals were found from initial screen drops. Crystal used for structure determination was grown in 20% PEG3350, 0.2 M Tri-Lithium Citrate in sitting drop setup.
Crystals grow to a mountable size after 1 days.
A mixture of 23% P3350, 8% P400 and 0.3M NaCl was used as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: