# ARFGAP1

**PDB:**3DWD

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**BC028233
**Entry Clone Source:**MGC AT50-C10
**SGC Clone Accession:**HPC039-A02
**Tag:**mgsshhhhhhssglvpr*gs, Thrombin cleavage tag
**Host:**BL21-CodonPlus(DE3)-V2R

## Construct

**Prelude:**
**Sequence:**

mgsshhhhhhssglvprgsMASPRTRKVLKEVRVQDENNVCFECGAFNPQWVSVTYGIWICLECSGRHRGLGVHLSFVRSVTMDKWK
DIELEKMKAGGNAKFREFLESQEDYDPCWSLQEKYNSRAAALFRDKVVALAEGREWSLES

**Vector:**pET28a-LIC (GI:145307000)

## Growth

**Medium:**Terrific Broth
**Antibiotics:**
**Procedure:**LEX Bubbling. The target protein was expressed in E. coli by inoculating 60 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin at 37 °reeC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 µC and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before they were harvested and flash frozen in liquid nitrogen and stored at -80 °C.

## Purification

**Procedure**
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 3 mL 50% Ni-NTA beads, and incubated at 4 °reeC for 1 hours. The supernatant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 15 mL washing buffers(contains 5mM, 30mM or 75 mM Imidazole separately). The protein bound to beads were eluted using 15 mL elution buffer once. The flow-through fractions washed using buffer containing 30mM, 75mM, 300mM Imidazole were collected and loaded onto Supderdex-75 gel filtration column. Eluted fractions were pooled and concentrated using amicon

centrifugal filter (m.w. cut-off 10,000 ). The purity of the proteins was higher than 85% judged by SDS-PAGE. High molecular weight bands were seen at 56 kDa-130kDa that cannot be separated using gel filtration.

## Extraction

**Procedure**
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer.
**Concentration:**66.6 mg/mL
**Ligand**
Zn**MassSpec:**Native: 16691.20, expected 16822.02, the N-terminal Met was removed when expressed in E.coli.
**Crystallization:**Crystallization was setup using sitting drops with Red Wings and SGC-I screens and also with in situ proteolytic treatment. The crystal used for data collection was grown in 3.5M Sodium Formate 0.1M Tris pH 8.5 and in the presence of 1:100 Chymotrypsin. The crystals grow to a mountable size within one week and shows 2D-plate shape. 2.0 M Li2SO4 or 4.2M NaCl were used as cryoprotectant.
*last updated by ytong 20080729*
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**