# ARL10C

**PDB:**2AL7

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**NM 018184
**Entry Clone Source:**MGC
**SGC Clone Accession:**
**Tag:**N-terminal His-tag with integrated thrombin protease site before the last Ser: MGSSHHHHHHSSGLVPR*GS
**Host:**E. coli BL21 (DE3)

## Construct

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

mgsshhhhhhssglvprgsKEEMELTLVGLQYSGKTTFVNVIASGQFSEDMIPTVGFNMRKVTKGNVTIKIWDIGGQPRFRSMWERY
CRGVNAIVYMIDAADREKIEASRNELHNLLDKPQLQGIPVLVLGNKRDLPNALDEKQLIEKMNLSAIQDREICCYSISCKEKDNIDI
TLQWLIQHSKSR

**Vector:**p28a-LIC

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**We prepared a seed culture by inoculating freshly transformed E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After overnight, the seed culture was 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 4.63. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20ºC in a 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 pH7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The eluate was dialyzed overnight against a buffer containing 10 mM Tris pH7.5, 0.5 M NaCl, 5% glycerol. The

protein concentration was estimated based on the Bradford assay. Five molar equivalents of GDP, 5 mM DTT 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 20 mg/ml. About 55 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 24000 rpm for 30 min and the supernatant was used for subsequent steps of purification.
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**Purified His-tagged ARL10C was crystallized using the sitting drop vapor diffusion method. Crystals grew in one day when the protein (20 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 30 % PEG 8000, 0.1 M Na Cacolidate, pH 6.0 and 0.2 M Na Acetate.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**