# RAB4B-GDP

**PDB:**2O52

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**NM_016154
**Entry Clone Source:**Codon Devices
**SGC Clone Accession:**
**Tag:**N-terminal hexahistidine tag
**Host:**E.coli. BL21 (DE3) codon(+) RIL

## Construct

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

mgsshhhhhhssglvprgsIWSDFLFKFLVIGSAGTGKSCLLHQFIENKFKQDSNHTIGVEFGSRVVNVGGKTVKLQIWDTAGQERF
RSVTRSYYRGAAGALLVYDITSRETYNSLAAWLTDARTLASPNIVVILCGNKKDLDPEREVTFLEASRFAQENELMFLETSALTGEN
VEEAFLKCARTILNKIDSGELDPERM

**Vector:**p28a-thrombin-lic

## Growth

**Medium:**Terrific Broth
**Antibiotics:**
**Procedure:**The target protein was expressed in E.coli. BL21 (DE3) coden (+) RIL containing the plasmid. The 100 ml overnight culture in Luria-Bertani medium was inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin and 50 µg/mL chloramphenicol at 37 ºC and grown to an OD600 ~ 3.0 in the SGC LEX bubbling system. The culture was induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.0 mM and grown overnight at 18 ºC. The culture was harvested with centrifugation. Pellets were flash frozen and stored at -80 ºC.

## Purification

**Procedure**
Column 1: Ni-NTA beads
Procedure: 2.5 ml of Ni-NTA suspension solution was added into 100 ml cell lysis supernatant solution. The mixture was shaken for 1 hour at 4 ºC. Beads were collected by centrifugation at 2500 rpm for 5 minutes. Beads were washed with 75 ml washing buffer, then collected by centrifugation. Protein was eluted with 15 ml elution buffer.

Column 2 : Size exclusion chromatography (Superdex 75 26/60)
The fractions eluted off the Ni-affinity column were applied to a Superdex 75 column (26/60) equilibrated in SEC buffer at a flow rate of 2.0 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

Protein concentration: Amicon ultra centrifugal filter with a 5kDa cut off in SEC-buffer

## Extraction

**Procedure**
Cells were thawed and resuspended in 100 mL binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5 mM imidazole) with 0.5% CHAPS (Sigma) and 1 mM phenylmethyl sulfonyl fluoride (PMSF), 0.5% (v/v) protease inhibitor cocktail (Sigma), 1 mM Benzamidine, 1600 units Benzonase (Sigma), and lysed with a microfluidizer. The lysate was centrifuged at 16000 rpm for 45 min and the supernatant was used for subsequent steps of purification. All the extraction steps were carried out at 4 ºC.
**Concentration:**
**Ligand**
**MassSpec:**
**Crystallization:**Crystals were obtained using the vapor diffusion method. The protein solution (17 mg/ml) contained a 5 fold molar of GDP and $MgCl_2$. 0.5 µl of the concentrated protein was mixed with 0.5 µl of a well solution containing 18% PEG8000, 0.2 M $Mg(OAc)_2$, 0.1 M NaCaco, pH 6.50. Crystals appeared after three days at 18 ºC.
**NMR Spectroscopy:**
**Data Collection:**Crystals were cryo-protected using a mixture of 50% mineral oil and 50% parotone, and flash frozen in liquid nitrogen. Diffraction data were collected at Rigaku FR-E with Rigaku R-AXIS IV++ detector to 2.2 Å.
**Data Processing:**