# Rab9B

**PDB:**2OCB

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**NM_016370
**Entry Clone Source:**Origene
**SGC Clone Accession:**HPC033-E07
**Tag:**N-terminal hexa histidine tag with thrombin cleavage site: mgsshhhhhhssglvprgs
**Host:**E.coli BL21 (DE3) codon plus RIL

## Construct

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

gsGKSLLLKVILLGDGGVGKSSLMNRYVTNKFDSQAFHTIGVEFLNRDLEVDGRFVTLQIWDTAGQERFKSLRTPFYRGADCCLLTF
SVDDRQSFENLGNWQKEFIYYADVKDPEHFPFVVLGNKVDKEDRQVTTEEAQTWCMENGDYPYLETSAKDDTNVTVAFEEAVRQVLA
VEEQLE

**Vector:** p28a-LIC

## Growth

**Medium:**Terrific Broth medium
**Antibiotics:**
**Procedure:**The target was expressed in E. coli by inoculating 100 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 and chloramphenicol at 37ºC. When OD600 was ~3.0, the culture was induced with 1mM IPTG and the temperature was reduced to 15ºC, and the cells were allowed to grow overnight. Cultures were harvested by centrifugation and the cell pellets were flash frozen and stored at -80ºC.

## Purification

**Procedure**
The frozen cell pellets were resuspended in 100 mL of 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 benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride), and 0.5% CHAPS. The cells were lysed by micro fluidizer at 20,000 psi. The lysate was centrifuged at 27,000xg for 30 min and the supernatant was passed through a DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto a 3 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer

at 4 ºC. The Ni-NTA column was washed with 150 mL of wash buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The His tag was cleaved overnight at 4ºC using 1 unit of thrombin (Sigma T9681) per milligram of protein by dialyzing the sample overnight against gel filtration buffer. The protein was further purified and desalted using a gel filtration column, Superdex 75 (26/60), which was pre-equilibrated with gel filtration buffer. Pooled protein fractions were concentrated using an Amicon Ultra centrifugal filter(5,000MWCO) to a final concentration of 33 mg/mL after the addition of 5mM GppNHp. Protein concentrations were measured using Bradford assay and the purity was >95% based on SDS-PAGE analysis.

## Extraction

### Procedure

**Concentration:**33 mg/mL
**Ligand**
GppNHp, Mg2+**MassSpec:**
**Crystallization:**Crystallization trials were set up using the sitting drop vapor diffusion method. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 2M NH4SO4, 0.2M NaCl and 0.1M HEPES pH7.5. Crystals reached a size of about 200 microns within a week at 18ºC.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**