# RAB3B

**PDB:**3DZ8

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**BC005035
**Entry Clone Source:**MGC AU35-H11
**SGC Clone Accession:**HPC085-A01
**Tag:**mhhhhhhssgrenlyfq*g
**Host:**BL21-CodonPlus(DE3)-V2R

## Construct

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

mhhhhhhssgrenlyfqgNFDYMFKLLIIGNSSVGKTSFLFRYADDTFTPAFVSTVGIDFKVKTVYRHEKRVKLQIWDTAGQERYRT
ITTAYYRGAMGFILMYDITNEESFNAVQDWATQIKTYSWDNAQVILVGNKCDMEEERVVPTEKGQLLAEQLGFDFFEASAKENISVR
QAFERLVDAICDKMSDS

**Vector:**pET28-mhl (GI:134105571)

## Growth

**Medium:**Terrific Broth
**Antibiotics:**
**Procedure:**LEX Bubbling. The target protein 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 25 µg/mL chloramphenicol 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 centrifuged at 16,000 rpm for 60 minutes and the supernatant was mixed with 3 mL 50% Ni-NTA beads, and incubated at 4 °reeC for 2 hours on a roller drum. The supernatant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 50 mL washing buffer twice. The protein bound to beads were eluted using 15 mL elution buffer. The flow-through was collected and loaded onto Supderdex-75 26/60 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 95% judged by SDS-PAGE.

## Extraction

**Procedure**
Frozen cells from 3.6L TB culture were thawed and resuspended in 400 mL extraction buffer with freshly added 0.5% CHAPS and 10U/mL Benzonase (Sigma Catalog # E1014, 250U/µL), and supplemented with 1mL protease inhibitor cocktail (SIGMA Catalog # P8849), and lysed using sonication at 10 seconds 50% duty cycle for 8 minutes at 120W.
**Concentration:**18.0 mg/mL
**Ligand**
GDP**MassSpec:**Native: 22155.29, expected 22154.94
**Crystallization:**Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. 2D-plate xtals were seen at condition SG04, SD11 several days after setup.
Crystal used for data collection was grown at 2.0M (NH4)2SO4, 0.2M NaAc, 0.1M HEPES pH 7.5, 5% MPD.
*Last updated by ytong 20080730*
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**