RAB5B

PDB:2HEI

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Synthetic DNA
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with thrombin cleavage site: mgsshhhhhhssglvprgs
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

gsASKICQFKLVLLGESAVGKSSLVLRFVKGQFHEYQESTIGAAFLTQSVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVV
YDITNQETFARAKTWVKELQRQASPSIVIALAGNKADLANKRMVEYEEAQAYADDNSLLFMETSAKTAMNVNDLFLAIAKKLPKSEP
QNLGG

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating glycerol stock of E. coli cells BL21-CodonPlus (DE-3)-RIL into 100 mL of Luria-Bertani medium. After overnight growth, all of the seeds were inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin and 50 µg/mL chloramphenicol at 37ºC and grown to an OD600 between 3-5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.0 mM and grown overnight at 18ºC in the SGC LEX bubbling system.

Purification

Procedure
The lysate was centrifuged at 15000 rpm for 45 min and the supernatant was loaded onto 5 mL HiTrap Ni-NTA column (Pharmacia Amersham) equilibrated with the same binding buffer at 4 ºC using peristaltic pump. The HiTrap Ni-NTA column was steply washed with 25 ml of binding buffer, 25 ml of binding buffer with 30 mM imidazole, and 25 ml of binding buffer with 50 mM imidazole. The His-tagged protein was eluted by linear gradient of imidazole from 50 mM to 500 mM in 50 ml. The eluted protein peak fractions detected by UV280 nm were combined and

further purified by gel filtration column superdex 75 with a buffer containing 20 mM HEPES pH 8.0, 500m M NaCl, 1 mM DTT. Protein peak fractions were combined, GDP (Sigma) was added to 5 times of the Rab5B protein in molarity, MgCl2 to the final concentration of 5 mM, DTT to the final concentration of 10 mM. The protein was concentrated using an Amicon Ultra centrifugal filter and the concentration for protein stock solution was estimated by Bradford to be 70.2 mg/mL.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were harvested and stored at -80 ºC before use. Cells were thawed and suspended in 150 mL the 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) and lysed with microfluidizer.
Concentration:30 mg/mL
Ligand
MassSpec:
Crystallization: Crystallization trials were set up using the hanging drop vapor diffusion method. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 25% w/v PEG 3350 0.1M sodium acetate. Crystals reached a size of about 50 microns within two to three days. Crystals used for data collection has a size of about 100 microns.
NMR Spectroscopy:
Data Collection:
Data Processing: