Rab14

PDB:2AED

Revision

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Entry Clone Accession:GI:19923483
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SGC Clone Accession:1E-01 (HPC006:F5)
Tag:N-terminal: His-tag with integrated thrombin protease site before the last Ser: MGSSHHHHHHSSGLVPRGS
Host:E. coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMATAPYNYSYIFKYIIIGDMGVGKSCLLHQFTEKKFMADCPHTIGVEFGTRIIEVSGQKIKLQIWDTA
GQGRFRAVTRSYYRGAAGALMVYDITRRSTYNHLSSWLTDARNLTNPNTVIILIGNKADLEAQRDVTYEEAKQFAEENGLLFLEASA
KTGENVEDAFLEAAKKIYQNIQ

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating glycerol stock of E. coli cells (BL21 DE3) into 80 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 at 37 degC and grown to an OD600 about 2 to 3. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.0 mM and grown overnight at 18 degC in the SGC LEX bubbling system.

Purification

Procedure
The supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 2 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). Protein eluted from the Ni-NTA column was further purified by a gel filtration column superdex G75 with a buffer containing 20 mM Tris pH 8.0, 0.15 M NaCl, 10 mM DTT. Protein peak fractions were combined, and 5 molar equivalents of GDP and 5 mM MgCl2 were added to the combined fraction before concentration. The protein was concentrated using an Amicon Ultra centrifugal filter to the final volume of 2.8 mL. The protein concentration estimated based on the extinction coefficient of the protein to be 19.0 mg/mL. About 53.1 mg of protein was obtained from 3.6 L of cell culture.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were harvested and stored at -80 ºC before use. Cells were thawed and suspended in 100 mL the binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole) with 0.5% CHAPS (Sigma) a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride) and lysed with microfluidizer. The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was used for subsequent steps of purification. All the extraction steps were carried out at 4degC.
Concentration:19.0 mg/mL
Ligand
Guanidine diphosphate (GDP)**MassSpec:**
Crystallization: Purified RAB14 was crystallized using the hanging drop vapor diffusion method at room temperature. Crystals grew in one day when the protein (19.0 mg/ml) was mixed with the reservoir solution in a 2:2 volume ratio, and the drop was equilibrated against a reservoir solution containing 20% PEG3350, 0.2M Calcium acetate, pH7.2. The crystals were flash frozen with the mother liquor with 15% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: