Rab2b

PDB:2A5J

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:GI:21361884
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site before the last Ser: MGSSHHHHHHSSGLVPRGS
Host:E. coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsYLFKYIIIGDTGVGKSCLLLQFTDKRFQPVHDLTIGVEFGARMVNIDGKQIKLQIWDTAGQESFRSIT
RSYYRGAAGALLVYDITRRETFNHLTSWLEDARQHSSSNMVIMLIGNKSDLESRRDVKREEGEAFAREHGLIFMETSAKTACNVEEA
FINTAKEIYRKIQQGLF

Vector:

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 37ºC 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 18ºC in the SGC LEX bubbling system. Extraction method 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 4ºC.

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 0.5 mL. The protein concentration estimated based on the extinction coefficient of the protein to be 39.1 mg/mL. About 19.5 mg of protein was obtained from 3.6 L of cell culture.

Extraction

Procedure

Concentration:
Ligand
MassSpec:
Crystallization: Purified RAB2 was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in one 3 days when the protein (39.1 mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 30% PEG5000 MME, 0.2M (NH4)2SO4, 0.1M MES, PH6.5. The crystals were flash frozen with the mother liquor with 15% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: