

RAB3D

PDB:2GF9

Revision

Revision Type:created

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Entry Clone Accession:BC016471

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-CodonPlus (DE-3)-RIL

Construct

Prelude:

Sequence:

MGSSHHHHHHSSGLVPRGSDYMFKLLLGNSVVGKTSFLFRYADDSTPAFVSTVGIDFKVKTVYRHDKRIKLQIWDTAGQERYRTI
TTAYYRGAMGFLMYDIANQESFAAVQDWATQIKTYSWDNAQVILVGNKCDLEDERVPAEDGRRLADDLGFEFFFEASAKENINVKQ
VFERLVDVICEKMNE

Vector:p28a-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 37°C and grown to an OD600 between 3-5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 0.5 mM and grown overnight at 18°C 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 5 ml HisTrap HP (Amersham) equilibrated with the same binding buffer at 4°C. The HisTrap HP 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 Tris pH 8.0, 500m M NaCl, 1 mM DTT. Protein peak fractions were combined, GDP (Sigma) 5 times of the Rab3D in molarity, and MgCl₂ to the final concentration of 5 mM MgCl₂ were added before concentration. The protein was concentrated using an Amicon Ultra centrifugal filter to the final volume of 0.8 mL. The protein concentration estimated by Bradford to be 30 mg/mL. About 75 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure

Cultures were centrifuged and the cell pellets were harvested and stored at -80 °C before use. Cells were thawed and suspended in 100 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. 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 4°C.

Concentration:80 mg/mL

Ligand

GDPMassSpec:

Crystallization:Purified RAB3D was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in 5 days when the protein (80 mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 20% PEG8K, 0.2M MgAc, 0.1M NaCaCo pH 6.5. The crystals were flash frozen with Parotone oil.

NMR Spectroscopy:

Data Collection:

Data Processing: