

Rab43

PDB:2HUP

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:GI:50234889

Entry Clone Source:Synthesized

SGC Clone Accession:

Tag:N-terminal: His-tag with integrated thrombin protease site before the last Ser:

MGSSHHHHHSSGLVPRGS

Host:E. coli BL21-CodonPlus (DE-3)-RIL

Construct

Prelude:

Sequence:

mgsshhhhhsglvprgsGDPDEQYDFLFKLVGVGDASVGKTCVQRFKTGAFSERQGSTIGVDFTMKTLEIQGKRVKLQIWDTAG
QERFRITQSYYRSANGAILAYDITKRSSFLSVPHIEDVRKYAGSNIVQLLIGNKSDLSELREVSLAEQSLAEHYDILCAIETSA
KDSSNVEEAFLRVATELIMRHGGPLFS

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 with 50 μ g/mL kanamycin and 50 μ g/mL chloramphenicol at 37°C and grown to an OD600 between 3 to 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 HiTrap Chelating HP (Amersham) equilibrated with the same binding buffer at 4 degC. The HiTrap Chelating HP column was washed with 25 ml binding buffer and then 25 ml binding buffer with 50 mM imidazole, and 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. The Gel filtration buffer contains 20 mM HEPES pH 7.8, 500m M NaCl, 1 mM DTT. Protein peak fractions were combined, GDP (Sigma) 5 times of the protein in molarity, MgCl₂ to the final concentration of 5 mM, and DTT to the final concentration of 5mM were added. The sample was set at room temperature for 30 min to allow the ligand binding before concentration. Protein sample was concentrated using an Amicon Ultra centrifugal filter to the final volume of 0.33 mL. The protein concentration estimated by Bradford to be 28.2 mg/mL. About 9.2 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 degC 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:

Ligand

MassSpec:

Crystallization: Purified Rab43 was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in 2 days when the protein (xxx mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 25% PEG8000, 0.2M NaCl, 0.1M Tris, pH8.5. The crystals were flash frozen with the mixture of paratone-N: mineral oil at 1:1 as the cryo solution.

NMR Spectroscopy:

Data Collection:

Data Processing: