

KIFC3 ATPase domain

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Revision

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Tag:His-tag integrated at the N-terminal of the protein was cleaved by thrombin protease, resulted in the addition of two residues (GS) attached to the first amino acid of the encoded protein

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

Construct

Prelude:

Sequence:

gsKGNIRVIARVRPVTKEDGEGPEATNAVTFDADDSIHLHKGPVSFELDKVFSPQASQQDVFQEVQALVTSCIDGFNCIFAY
GQTGAGKTYTMEGTAENPGIDQRALQLLFSEVQEKAQDWETITVSAEIQYNEVRDLLGKEPQEKLEIRLCPDGSQLYVPGLTEF
QVQSVDDINKVFEFGHTNRTEFTNLNEHSSRSHALLIVTVRGDCSTGLRTTGKLNLVDLAGSERVGKSGAEGSRLREAQHINKSL
SALGDVIAALRSRQGHVPFRNSKLTYLLQDLSGDSKTLMVQVSPVEKNTSETLYSLKFAERVRSVEL

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 μ g/mL of kanamycin and 50 μ g/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 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. Thrombin protease was added to the eluted protein and the protease digestion reaction was carried out at 4C. After 18 hours, almost all protein was His-tag removed as observed on SDS-PAGE. The his-tag removed protein sample was further purified by gel filtration column superdex 75. The Gel filtration buffer contains 20 mM HEPES pH 7.5, 500m M NaCl, 1 mM DTT. Protein peak fractions were combined, ADP (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 0.53 mL. The protein concentration estimated by Bradford to be 69.67 mg/mL. About 36.93 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: 69.67 mg/mL

Ligand

MassSpec:

Crystallization: Purified KIFC3 ATPase domain was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in 2 days when the protein (35 mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 1.2M sodium citrate, 0.1M Hepes, pH7.5. The crystals were flash frozen with 1.2M sodium citrate, 20% glycerol, 0.1M Hepes, pH7.5 as the cryo solution.

NMR Spectroscopy:

Data Collection:

Data Processing: