KIF2C ATPase domain

PDB:2HEH

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:5803082
Entry Clone Source:MGC
SGC Clone Accession:
Tag: His-tag with integrated thrombin protease site before the last Ser:
MGSSHHHHHHSSGLVPRGS
Host:E. coli BL21-CodonPlus (DE-3)-RIL

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsSFPNWEFARMIKEFRATLECHPLTMTDPIEEHRICVCVRKRPLNKQELAKKEIDVISIPSKCLLLVHE
PKLKVDLTKYLENQAFCFDFAFDETASNEVVYRFTARPLVQTIFEGGKATCFAYGQTGSGKTHTMGGDLSGKAQNASKGIYAMASRD
VFLLKNQPCYRKLGLEVYVTFFEIYNGKLFDLLNKKAKLRVLEDGKQQVQVVGLQEHLVNSADDVIKMIDMGSACRTSGQTFANSNS
SRSHACFQIILRAKGRMHGKFSLVDLAGNERGADTSSADRQTRMEGAEINKSLLALKECIRALGQNKAHTPFRESKLTQVLRDSFIG
ENSRTCMIATISPGISSCEYTLNTLRYADRVKELSPHSG

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 µg/mL kanamycin and 50 µg/mL chloramphenicol at 37ºC 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 18ºC 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 4ºC. 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 6.8, 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 0.5 mL. The protein concentration estimated by Bradford to be 122.15 mg/mL. About 61 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 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 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 4ºC.
Concentration:122.15 mg/mL.
Ligand
MassSpec:
Crystallization: Purified KIF2C ATPase domain was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in 2 days when the protein (50.7 mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 40% PEG400, 0.2M NaCl, 0.1M HEPES, pH7.5. 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: