KIF3B

PDB:3B6U

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_004789
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal hexahistidine tag
Host:E.coli. BL21 (DE3) codon(+) RIL

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgSSESVRVVVRCRPMNGKEKAASYDKVVDVDVKLGQVSVKNPKGTAHEMPKTFTFDAVYDWNAKQFELYD
ETFRPLVDSVLQGFNGTIFAYGQTGTGKTYTMEGIRGDPEKRGVIPNSFDHIFTHISRSQNQQYLVRASYLEIYQEEIRDLLSKDQT
KRLELKERPDTGVYVKDLSSFVTKSVKEIEHVMNVGNQNRSVGATNMNEHSSRSHAIFVITIECSEVGLDGENHIRVGKLNLVDLAG
SERQAKTGAQGERLKEATKINLSLSALGNVISALVDGKSTHIPYRDSKLTRLLQDSLGGNAKTVMVANVGPASYNVEETLTTLRYAN
RAKNIKNKPRVNEDPKDALLREFQ

Vector:p28a-mhl-tev

Growth

Medium: Terrific Broth medium
Antibiotics:
Procedure: We prepared the seeds by inoculating glycerol stock of E. coli cells BL21-CodonPlus (DE-3)-RIL containing the plasmid into 200 mL of Luria-Bertani medium. After overnight growth, all of the seeds were inoculated into 6 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin and 50 µg/mL chloramphenicol at 37 ºC 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 ºC in the SGC LEX bubbling system.

Purification

Procedure
Column 1: Ni-NTA beadsBuffers: Wash buffer 1: 1 X PBS, pH 7.5, 0.5 M NaCl, 30 mM Imidazole, 1 mM TCEP. Elution buffer: 1 X PBS, 0.5 M NaCl, 250 mM Imidazole, pH 7.5, 1 mM TCEP, 2 mM MgCl2.Procedure: 1 ml of Ni-NTA suspension solution was added into 80 ml cell lysis supernatant solution. The mixture was shaken for 1 hour at 4 ºC. Beads were collected with centrifuge at 2500 rpm, 5 minutes. Each fraction of beads was washed with 100 ml washing

buffer, then collected with centrifuge. Protein was eluted with 15 ml elution buffer. Column 2 : Size exclusion chromatography (Superdex 75 26/60)SEC-Buffers: 20 mM Hepes, pH 7.5, 500 mM NaCl, 1 mM TCEP. The fractions eluted of the Ni-affinity chromatography applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 2.0 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were harvested and stored at -80 ºC before use. Cells were thawed and suspended in 500 mL binding buffer (1 X PBS, 0.5 M NaCl, 5 mM imidazole, pH 7.5) with 0.5% CHAPS (Sigma), 0.5% (v/v) protease inhibitor cocktail (Sigma), 1 mM Benzamidine, 1600 units Benzonase (Sigma), and lysed with microfluidizer. The lysate was centrifuged at 16000 rpm for 60 min and the supernatant was used for subsequent steps of purification. All the extraction steps were carried out at 4 ºC.
Concentration:28 mg/ml
Ligand
ADP**MassSpec:**
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 28 mg/ml containing 5 molar fold of ADP and MgCl2. 2 µl of the concentrated protein mixed with 2 µl of a well solution containing 30% PEG 3350, 0.1 M Tris-HCl, 0.2M Lithium sulfate, pH 8.5. Crystals appeared after two days at 18 °C.
NMR Spectroscopy:
Data Collection:
Data Processing: