FTSJ2

PDB:2NYU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:7019377
Entry Clone Source:MGC
SGC Clone Accession:FTSJ2_11::C4S:F8-APC046
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

gsSYRsRSAFKLLEVNERHQILRPGLRVLDCGAAPGAWSQVAVQKVNAAGTDPSSPVGFVLGVDLLHIFPLEGATFLCPADVTDPRT
SQRILEVLPGRRADVILSDMAPNATGFRDLDHDRLISLCLTLLSVTPDILQPGGTFLCKTWAGSQSRRLQRRLTEEFQNVRIIKPEA
SRKESSEVYFLATQYHGRKGTVKQ

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:FTSJ2 protein was expressed in E.coli BL21 (DE3) codon plus RIL in 1L Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cells were grown at 37oC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation at ~75000 x g for 60 minutes. The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES, pH 7.4, containing 500 mM NaCl, 50 mM imidazole and 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM PIPES buffer, pH 6.5, and 250 mM NaCl, at flow rate 4 ml/min. 20 units of Thrombin (Sigma) was added to combined fractions containing FTSJ2 and incubated overnight at 4oC. The protein was further purified to

homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 30 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For purification, the cell paste from 1L of cells was thawed and resuspended in lysis buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:59 mg/ml
Ligand
MassSpec: The expected mass for FTSJ2 is 21826.01 Da, measured mass is 21826.2355 Da.
Crystallization: Purified FTSJ2 protein was complexed with S-adenosyl-L-methionine (SAM) (Sigma) at 1:10 molar ratio of protein:SAM and crystallized using sitting drop vapor diffusion method at 20 °C by mixing the protein solution with the reservoir solution containing 20% PEG 3350, 0.2 M potassium phosphate.
NMR Spectroscopy:
Data Collection:
Data Processing: