MYST1

PDB:2GIV

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:14149875
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsTKVKYVDKIHIGNYEIDAWYFSPFPEDYGKQPKLWLCEYCLKYMKYEKSYRFHLGQCQWRQPPGKEIY
RKSNISVHEVDGKDHKIYCQNLCLLAKLFLDHKTLYFDVEPFVFYILTEVDRQGAHIVGYFSKEKESPDGNNVACILTLPPYQRRGY
GKFLIAFSYELSKLESTVGSPEKPLSDLGKLSYRSYWSWVLLENLRDFRGTLSIKDLSQMTSITQNDIISTLQSLNMVKYWKGQHVI
CVTPKLVEEHLKSAQYKKPPITVDSVCLKWAPPK

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:MYST1 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation and passing through 20-ml DE52 column equilibrated in 20 mM HEPES, pH 7.4, containing 1 M NaCl and 5% glycerol. The lysate was loaded onto 10 ml Chelating Sepharose column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES buffer, pH 7.4, containing 0.5 M NaCl , 50 mM imidazole, 5% glycerol and 0.1% CHAPS, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 0.5 M NaCl, 250 mM imidazole, 5% glycerol, 0.1 % CHAPS). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with

20 mM HEPES buffer, pH 7.4, and 0.5 M NaCl, at flow rate 4 ml/min. Purification yield was 8.2 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For the purification the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol, 0.1% CHAPS) 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:6.8 mg/ml
Ligand
MassSpec: The expected mass for MYST1 is 34364.5 Da, measured mass is 34275.5 Da.
Crystallization: Purified MYST1 was complexed with acetylcoenzyme A (AcCoA, Sigma) at 1:10 molar ratio of protein:AcCoA and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1µl of the protein solution with 1 µl of the reservoir solution containing 20% PEG3350, 0.2 M CaCl2.
NMR Spectroscopy:
Data Collection:
Data Processing: