MYST3

PDB:2OZU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_006757
Entry Clone Source:MGC
SGC Clone Accession:MYST3_01:D12-APC027
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsGVTGPPDPQVRCPSVIEFGKYEIHTWYSSPYPQEYSRLPKLYLCEFCLKYMKSRTILQQHMKKCGWFH
PPANEIYRKNNISVFEVDGNVSTIYCQNLCLLAKLFLDHKTLYYDVEPFLFYVLTQNDVKGCHLVGYFSKEKHCQQKYNVSCIMILP
QYQRKGYGRFLIDFSYLLSKREGQAGSPEKPLSDLGRLSYMAYWKSVILECLYHQNDKQISIKKLSKLTGICPQDITSTLHHLRMLD
FRSDQFVIIRREKLIQDHMAKLQLNLRPVDVDPECLRWTPVI

Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:MYST3 was expressed in E.coli BL21 (DE3) codon plus RIL in 4L Terrific Broth (TB) in the presence of 50 microG/mL of kanamycin. Cell were grown at 37degC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, incubated overnight at 15degC.

Purification

Buffers
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Procedure
The crude extract was cleared by centrifugation. 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 500 mM NaCl , 50 mM imidazole, 5% glycerol and 0.1% CHAPS, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 250 mM imidazole, 5% glycerol, 0.1 % CHAPS). Eluted MYST3 was treated

with iodoacetamide. The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM HEPES buffer, pH 7.4, and 500 mM NaCl, at flow rate 4 ml/min. Purification yield was 1.4 mg of the protein per 1L of culture

Extraction

Buffers

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Procedure

Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80degC. 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 Beta-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:5 mg/mL

Ligand

MassSpec: The expected mass for MYST3 is 35452.20 Da, measured mass is 35577.0652 Da.

Crystallization: Purified MYST3 was complexed with acetylcoenzyme A (AcCoA, Sigma) at 1:10 molar ratio of protein:AcCoA and crystallized using the hanging drop vapor diffusion method at 20degC by mixing 1microL of the protein solution with 1 µl of the reservoir solution containing 12% PEG 5000, 0.1 M BisTris, pH 6.0.

NMR Spectroscopy:

Data Collection:

Data Processing: