Cp-LSm5: Cryptosporidium parvum U6-snRNA-associated Sm-like protein

PDB:2FWK

Revision

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Entry Clone Accession:cgd7_4590
Entry Clone Source:*Cryptosporidium parvum* strain Iowa genomic DNA
SGC Clone Accession: Cp-PF14_0411; plate MR:B12
Tag: His6-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:
Sequence:

gsSYKVNYMSETPANKSQGGSNQKGGNIILPLALIDKCIGNRIYVVMKGDKEFSGVLRGFDEYVNMVLDDVQEYGFKADEEDISGGN
KKLKRVMVNRLETILLSGNNVAMLVPGGDPDSFNFS

Vector:pET28a-LIC

Growth

Medium: Terrific Broth (TB)
Antibiotics:50 microG/mL kanamycin and 25 microG/mL chloramphenicol
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 degC for 3 hours. The culture was then transferred into 1.8 L of above-specified growth medium with Antibiotics and 0.3 mL of antifoam (Sigma) in a 2L bottle and cultured using the LEX system to an OD600 of ~5, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure
The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and subsequently onto a 1.0-2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1-1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. EDTA was immediately added to the elution fraction to 1 mM; and DTT was added to 1-5 mM after approximately 15 more minutes.

The eluted protein was applied to a Sephadex S75 26/60 gel filtration column pre-equilibrated with Gel Filtration Buffer. The collected fractions corresponding to the eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore).

The His6-tag was cleaved with thrombin overnight at 4 degC in the presence of 5 mM CaCl2. The cleaved sample was applied to a 2.5 mL Ni-NTA column pre-equilibrated with Binding Buffer 2. Imidazole was added to the cleaved Cp-LSm5 sample to 15 mM and applied to the Ni-NTA column. The flow-through was collected; and the column was rinsed was an additional 5 mL of Binding Buffer 2. These fractions were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore).

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF). Resuspended pellets stored at -80 degC were thawed overnight at 4 degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5% CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18,000 psi. The cell lysate was centrifuged at ~75,000 x g for 20 minutes at 10 degC.
Concentration:24.5 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means of hanging drop vapor diffusion in a 24-well Linbro plate. The plate was set with 1.5 microL protein and 1.5 microL buffer in each drop, and 500 microL reservoir volume per well. Crystals grew after several day in 1.05 M NaCitrate, 100 mM BisTris, pH 6.5 at 18 degC.
NMR Spectroscopy:

Data Collection:
Data Processing: