

Cp-NTF2: Cryptosporidium parvum nuclear transport factor 2

PDB:1ZO2

Revision

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Entry Clone Accession:[cgd4_590](#)

Entry Clone Source:Cryptosporidium parvum strain Iowa genomic DNA

SGC Clone Accession:CP-PF14_0122; plate MR:C6

Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHSSGLVPR*GS

Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:

Sequence:

gsDQSINLNQFDQIGKQFVQHYYQTFQTNRPALGGLYGPQS_mLTWEDTQFQGQANIVNKFNSLNQQRVQFEITRVDCQPSPNNGSI
VFVTGDVRIDDGQPLKFSQVNLMPSGNGGFMI_fNNDLFRLNLG

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 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 ~1, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure

The cleared cell lysate was loaded onto a column containing 10 g DE-52 resin (Whatman) anion exchangeresin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer), and then directly onto a 2.5 mL Ni-NTA (Qiagen) column at approximately 1.5 mL/min. When all the lysate was loaded, 20 mL of Binding Buffer was added to the DE52 column. Then the Ni-NTA column was washed with 200 mL of Wash Buffer. After washing, the protein was eluted from the

Ni-NTA column with 15 mL of Elution Buffer. EDTA was added immediately to 1 mM.DTT was added to 5 mM 15 minutes later.

The eluted protein was applied to a Sephadex S200 26/60 gel filtration column (GE Healthcare) 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 from Millipore (10 kDa cutoff). The concentrated sample was flash frozen in N₂(l) and stored at -80 degC.

The thawed sample was incubated with thrombin overnight at 4 degC in the presence of 5 mM CaCl₂ to cleave the His-tag. The cleaved sample was applied to a 2.5 mL Ni-NTA column pre-equilibrated with 10 mM HEPES, pH 7.5, 500 mM NaCl and 15 mM imidazole. Imidazole was added to the cleaved sample to 15 mM and applied to the Ni-NTA column. The flow-through was collected and the column was rinsed with an additional 5 mL of 10 mM HEPES, pH 7.5, 100 mM NaCl and 15 mM imidazole. These fractions were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device from Millipore (10 kDa cutoff). The concentrated protein was flash frozen in N₂(l) in 150 microL aliquots and stored at -80 degC.

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: 7 mg/mL

Ligand

MassSpec:

Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a Linbro plate. The plate was set with 1 microL protein (20 mg/mL) and 1 microL buffer in each drop, and 500 microL reservoir volume per well. Crystals emerged in 16% PEG 3350 and 200 mM NH₄PO₄ at 18 degC overnight.

NMR Spectroscopy:

Data Collection:

Data Processing: