

Cp-NHP2: Cryptosporidium parvum High Mobility Like Non-Histone Protein 2

PDB:2AIF

Revision

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Entry Clone Accession:cgd6_3850

Entry Clone Source:Cryptosporidium parvum strain Iowa genomic DNA

SGC Clone Accession:PY02252; 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:

mgsshhhhhhsglvprgsSQNEASEDTGFNPKAFLASPDLNKIINLVQQACNYKQLRKANEATKALNRGIAEIVLLAADAEPLEILLHLPLVCEDKNTPYVFVRSKVALGRACGVSRPVIAAAITSKGSSLSSQITELKDQIEQILV

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 cell lysate was loaded onto a DE52 (Whatman) column packed with 10 g of resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer), and subsequently onto a 2.5 mL Ni-NTA 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 at 2 Å 2.5 mL/min. 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 then added to 1 mM 15 minutes later.

The eluted protein was applied to a Sephadex S200 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 concentrated sample was flash frozen in N2(l) and stored at -80 degC. From the thawed sample, the His-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. 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 (Millipore) with a 5kD 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: 30 mg/mL for uncleaved protein and 19.6 mg/mL for cleaved protein

Ligand

MassSpec:

Crystallization: The protein was crystallized by means of sitting drop vapor diffusion in an Intelli-Plate. The plate was set with 0.5 microL cleaved protein and 0.5 microL buffer in each drop, and 100 microL reservoir volume per well. Crystals grew overnight in 1.4 M sodium citrate and 100 mM HEPES, pH 7.5 at 18 degC.

NMR Spectroscopy:

Data Collection:

Data Processing: