

Cp-Vsp29: *Cryptosporidium parvum* vacuolar protein sorting 29

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Revision

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Entry Clone Accession:cgd7_2060

Entry Clone Source:Cryptosporidium parvum strain Iowa genomic DNA

SGC Clone Accession:CP-PF14_0064; plate MS:A10

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

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

Construct

Prelude:

Sequence:

mgsshhhhhssglvprgssSTDFGDLVLLIGDLKIPYGAKELPSNFRELLATDKINYVLCTGNVCSQEYVEMLKNITKNVYIVSGD
LDSAIFNPDPESNGVFPEYVVVQIGEFKIGLMHGNQVLPWDDPGSLEQWQRRLCDILVTGHTHKLRFVEKNGKFLNPGTATGAFS
ALTPDAPPSFMLMALQGNKVVLVYDLRDGKTNVAMSEFSK

Vector:pET28a-LIC

Growth

Medium:Terrific Broth (TB)

Antibiotics:

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 2.5, 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 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 3 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 to 7 mg/mL using a 15 mL Amicon Ultra centrifugal filter device (5 kD cutoff). The concentrated sample was flash frozen in N₂(l) 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: 27 mg/mL

Ligand

MassSpec:

Crystallization: The protein was crystallized by means of hanging drop vapor diffusion in a Linbro plate. The plate was set with 1 microL protein (27 mg/mL) and 0.75 microL buffer in each drop, and 500 microL reservoir volume per well. Crystals grew in 22% (w/v) polyethylene glycol (PEG) 4000, 100 mM sodium citrate and 200 mM ammonium acetate at pH 5.6 and 18 degC in 5 days.

NMR Spectroscopy:

Data Collection:

Data Processing: