CpHSP70

PDB:3L6Q

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NCBI: cgd2_20
Entry Clone Source:
SGC Clone Accession:cgd2_20:S12-N394:F5
Tag:N-terminal tag: mhhhhhhssgrenlyfqg
Host:BL21-(DE3)-V2R-pRare2.

Construct

Prelude:
Sequence:

GPAIGIDLGTTYSCVGVWRNDTVDIVPNDQGNRTTPSYVAFTETERLIGDAAKNQVARNPENTVFDAKRLIGRKFDDQAVQSDMTHW
PFKVVRGPKDKPIISVNYLGEKKEFHAEEISAMVLQKMKEISEAYLGRQIKNAVVTVPAYFNDSQRQATKDAGAIAGLNVMRIINEP
TAAAIAYGLDKKGTGERNVLIFDLGGGTFDVSLLTIEDGIFEVKATAGDTHLGGEDFDNRLVEFCVQDFKRKNRGMDLTTNARALRR
LRTQCERAKRTLSSSTQATIELDSLYEGIDYSVAISRARFEELCADYFRATLAPVEKVLKDAGMDKRSVHDVVLVGGSTRIPKVQAL
IQEFFNGKEPCKAINPDEAVAYGAAVQAAILNGE

Vector:p15-mhl

Growth

Medium:TB
Antibiotics:
Procedure: Cryptosporidium parvum Hsp70, cgd2_20, was expressed in *E. coli* BL21(λDE3) V2R pRare2 in TB growth media in the presence of carbenicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL, respectively). A single colony was inoculated into 25 mL of LB with of carbenicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37degC. Then the culture was transferred into 900 mls of TB with 100 microgram/mL Carbenicillin and 34 microgram/ml chloramphenicol , 0.3 mL of antifoam (Sigma), 9 mls of 0.83 M MgSO4 and trace elements in a 1L bottle and cultured using the LEX system to an OD600 of 5, cooled to 15 °C, 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 2mL 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. 1 mM TCEP and 1 mM EDTA was added to the eluted cgd2_20.

The sample was then loaded onto a superdex 200 gel filtration column. The eluted protein (in 10 mM Hepes, pH 7.5 and 500 mM NaCl) was concentrated using a 15 ml Amicon Ultra centrifugal filter device (Millipore) with a 10 kDa cutoff. PP-HSP70 (cgd2_20) was concentrated to 9.8 mg/ml and stored at 4 degC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 2 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine 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 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 degC.
Concentration:9.8 mg/ml
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 degC in 30% PEG 400, 0.2 M Ammonium sulfate, 0.1 M Hepes pH 7.5 with ligand 4 mM MgCl2, 2 mM TCEP using the Sitting drop method.
NMR Spectroscopy:
Data Collection:
Data Processing: