PF14_0020

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Revision

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Entry Clone Accession:PF14_0020
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SGC Clone Accession:PF14_0020:K80-D440:B6
Tag:N-terminal tag: mhhhhhhssgrenlyfqg
Host:BL21-(DE3)-V2R-pRare

Construct

Prelude:
Sequence:

KLTDPLYIKKICLEKVHDWSRCNEDDVCVNQILSGLTNQLFEVSIKEDTAIEYRITRRHVLFRIYGKDVDALYNPLSEFEVYKTMSK
YRIAPLLLNTFDGGRIEEWLYGDPLSIDDLKNKSILVGIANVLGKFHTLSRKRHLPEHWDKTPCVFKMMDRWRLAVSNYKNLDKVTL
DINKYIQESHKFLKFIKIYTQIENIANDIVFCHNDLQENNIMNTNKCLRLIDFEYSGYNFLSADIANFFIETTIDYSYNAYPFFIIN
KKNYISYESRILFVTTYLSKYLDDSTAASDQDIIDQFLEAIEVQALGLHLIWAFWSIIRGYQTKSYNEFDFFLYAKERLKMYDEQKQ
YLMSKNIIKDYDD

Vector:p15-mhl

Growth

Medium:TB
Antibiotics:
Procedure: Plasmodium falciparum Pf-ChK (PF14_0020) was expressed in E. coli BL21(λDE3) V2RpRare2 in Terrific Broth (TB) in the presence of kanamycin/chloramphenicol (50 microgram/mL and 25 microgram/mL, respectively). A single colony was inoculated into 10 mL of LB with of kanamycin/chloramphenicol (50 microgram/mL and 25 microgram/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 50 mL of TB with 50 microgram/mL kanamycin in a 250 mL shaking flask and incubated at 37 °C for 3 hours. Then the culture was transfer into 1.8 L of TB with 50 microgram/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 > 5, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

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. 5 mM DTT and 1 mM EDTA was added to the eluted Pf-ChK (PF14_0020).

The Pf-Chk (PF14_0020) His-tag was cleaved with Tev protease overnight at 4 ºC in the presence of 1 mM TCEP (Tris(2-Carboxyethyl) phosphine Hydrochloride). 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 Pf-ChK (PF14_0020) 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 10 mM HEPES, pH 7.5, 100 mM NaCl, and 15 mM imidazole.

The His tag cleaved 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 5 kDa cutoff. Pf-Chk (PF14-0020) was concentrated to 43.3 mg/ml and flash frozen in N2(l) and stored at -80C. Proein was diluted to 21.6 mg.ml for crystallization.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 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:43.3 mg/ml.
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 degC in 20% Peg 8000, 0.2 M NaCl, 0.1 M Hepes pH 7.5, 5% MPD using the Sitting drop method. 2 mM TCEP, 2 mM ADP, 4 mM MgCl2,2 mM Phosphoethanolamine added directly to the concentrated protein immediately prior to setting up the crystallization plate
NMR Spectroscopy:
Data Collection:
Data Processing: