PYRK1

PDB:3KHD

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:PFF1300w:A10-E511:H10
Tag:N-terminal tag: mhhhhhhssgrenlyfqg
Host:BL21-(DE3)-V2R-pRare2

Construct

Prelude:
Sequence:

AAGASMQSAANITLRQILEPNNVNLRSKKTHIVCTLGPACKSVETLVKLIDAGMDICRFNFSHGSHEDHKEMFNNVLKAQELRPNCL
LGMLLDTKGPEIRTGFLKNKEVHLKEGSKLKLVTDYEFLGDETCIACSYKKLPQSVKPGNIILIADGSVSCKVLETHEDHVITEVLN
SAVIGERKNMNLPNVKVDLPIISEKDKNDILNFAIPMGCNFIAASFIQSADDVRLIRNLLGPRGRHIKIIPKIENIEGIIHFDKILA
ESDGIMIARGDLGMEISPEKVFLAQKLMISKCNLQGKPIITATQMLESMTKNPRPTRAEVTDVANAVLDGTDCVMLSGETAGGKFPV
EAVTIMSKICLEAEACIDYKLLYQSLVNAIETPISVQEAVARSAVETAESIQASLIIALTETGYTARLIAKYKPSCTILALSASDST
VKCLNVHRGVTCIKVGSFQGTDIVIRNAIEIAKQRNMAKVGDSVIAIHGIKEEVSGGTNLMKVVQIE

Vector:p15-mhl

Growth

Medium:TB
Antibiotics:
Procedure:*Plasmodium falciparum* PFF1300w 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 34microgram/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 degC, 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 PFF1300w.
TEV protease was then added to the protein to cleave the His-tag and the sample was dialysed overnight in 10 mM Hepes, pH 7.5, 500 mM NaCl, 1 mM DTT. The following day, imidazole was added to the sample at 15 mM final concentration, then the sample was loaded onto a 2 ml Ni-NTA (Qiagen) column pre-equilibrated with binding buffer + 15 mM imidazole, pH 7.5. The sample was allowed to bind to the nickel resin for 30 minutes after which the flow through containing the the cut protein was collected.
The protein sample with the tag removed 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. The protein was then buffer exchanged , to remove the NaCl, by back diluting to 5 ml with buffer containing 10 mM Hepes, pH 7.5, 100 mM KCl and dialysed in 10 mM Hepes, pH 7.5, 100 mM KCl, 1 mM DTT. The protein was reconcentrated to 6.8 mg/ml using a 15 ml Amicon Ultra centrifugal filter device (Millipore) with a 10 kDa cutoff, then stored at 4 º C.

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:
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 degC in 10% PEG8000, 0.1 M Tris pH 8.5 using the Sitting drop method. 2 mM NaPyruvate,4 mM MgAcetate, 2 mM AMPPNP and 2 mM TCEP were used as ligand.
NMR Spectroscopy:
Data Collection:
Data Processing: