PFD0660w

PDB:3EOZ

Revision

Revision Type:created
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Entry Clone Accession:PFD0660w:N100-F295:B6
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal tag: mhhhhhhssgrenlyfqg
Host:BL21-(DE3)-V2R-pRare2.

Construct

Prelude:
Sequence:

NTTKHIILVRHGQYERRYKDDENSKRLTKEGCKQADITGKKLKDILNNKKVSVIYHSDMIRAKETANIISKYFPDANLINDPNLNEG
TPYLPDPLPRHSKFDAQKIKEDNKRINKAYETYFYKPSGDEDEYQLVICHGNVIRYFLCRALQIPLFAWLRFSSYNCGITWLVLDDE
GSVVLREFGSVSHLPFESVTYF

Vector:p15-mhl

Growth

Medium:M9
Antibiotics:
Procedure: Plasmodium falciparum Pf-PGAM2 (PFD0660w) was expressed in E. coli BL21(λDE3) V2R pRare2 in M9 SeMet High-Yield growth media (Medicilon) in the presence of kanamycin/chloramphenicol (50 µg/mL and 34 µg/mL, respectively). A single colony was inoculated into 50 mL of LB media with of kanamycin/chloramphenicol (50 µg/mL and 34 µg/mL, respectively). in a 250mL flask and incubated with shaking at 250 rpm overnight at 37 °C. The 50ml over night culture, was centrifuged at 2000 rpm and the cell pellets were washed with M9 SeMet High yield growth media. The resuspended pellet was added to 1.8 L of M9 SeMet High-Yield growth media with 50 microgram/mL kanamycin, 34 microgram/ml chloramphenicol and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 =1.5 . The inhibitory amino acid cocktail and SeMet was then added to the cultures and the temperature was lowered to 15 °C. Twenty minutes later, the cultures were 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. 1 mM DTT and 1 mM EDTA was added to the eluted Pf-PGAM2(PFD0660w).

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: 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 5 kDa cutoff. Pf-PGAM2 (PFD0660w) was concentrated to 12.4 mg/ml and stored at 4 degC.
Ligand
MassSpec:
Crystallization: Hanging drop using Nextal drop guard plates; 2 M $(NH_4)_2SO_4$, 0.2 M NaAcetate, 0.1 M Hepes pH 7.5, 5 % MPD, 25% glycerol, then paratone. 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: