Pf-GUK: Plasmodium falciparum guanylate kinase (PFI1420w)

PDB:1Z6G

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PFI1420w
Entry Clone Source: Pf3D7 genomic DNA
SGC Clone Accession:PFI1420w; plate 2001:A8
Tag: His-tag with integrated thrombin protease site: mgsshhhhhhssglvprgs
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMNNIYPLVICGPSGVGKGTLIKKLLNEFPNYFYFSVSCTTRKKREKEKEGVDYYFIDKTIFEDKLKNE
DFLEYDNYANNFYGTLKSEYDKAKEQNKICLFEMNINGVKQLKKSTHIKNALYIFIKPPSTDVLLSRLLTRNTENQEQIQKRMEQLN
IELHEANLLNFNLSIINDDLTLTYQQLKNYLLNSYIHLNNHTRN

Vector:pET28a-LIC

Growth

Medium: Terrific broth (TB)
Antibiotics:50 microG/mL kanamycin and 25 microG/mL chloramphenicol
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics Mixture and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics Mixture 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 Mixture and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of ~1, 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 DE52 (Whatman) column packed with 10 g of resin and subsequently onto a 2.5 mL Ni-NTA 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 each Ni-NTA column was washed with 200 mL of Wash Buffer at 2 - 2.5 mL/min. After washing, the protein was

eluted from the Ni-NTA column with 15 mL of Elution Buffer. EDTA was added immediately to 1 mM and DTT was added to 1 mM 15 minutes later. The eluted protein was dialyzed overnight at 4 degC against 50 mM HEPES, 500 mM NaCl, 250 mM imidazole and 5% glycerolin a dialysis bag from Pierce. The next morning, the protein was transferred out of the dialysis bag and concentrated using 15 mL Amicon Ultra centrifugal filter device from Millipore (5 kD cutoff).

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 ~75000 x g for 20 minutes at 10 degC.
Concentration:35 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by sitting drop vapor diffusion in a 96-well Intelli-Plate. The plate was set with 1.0 microL protein (35 mg/mL) and 1.0 microL buffer in each drop, and 100 microL reservoir volume per well. Crystals emerged in 0.1 M Hepes, 2 M ammonium sulphate at pH 7.5 and 20 degC in 2 weeks.
NMR Spectroscopy:
Data Collection:
Data Processing: