PVX_092040

PDB:3EZ3

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession: Pv092040
Entry Clone Source: Plasmodium vivax Salvador I genomic DNA
SGC Clone Accession: Pv-PF11_0295; plate MAC01Q:A12
Tag:N-terminal: His6-tag with integrated TEV protease site: mhhhhhhssgrenlyfq*g
Host:*E. coli* BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqgMKETNSEEADSGLAFFRNMYDKYRDAFLSHLNEYSLEEEIKEHISKYYKLLFDYNCLGGKNNRGIL
VILIYEYVKNRDINSSEWEKAACLAWCIEILQAAFLVADDIMDKGEMRRNKYCWYLLKDVETKNAVNDVLLLYNSIYKLIEIYLRNE
SCYVDVIATFRDATLKTIIGQHLDTNIFSDKYSDAHREIDVNNINVPEQPVIDINMINFGVVYKNIVIHKTAYYSFFLPIVCGMLLAG
IAVDNLIYKKIEDISMLMGEYFQIHDDYLDIFGDSTKTGKVGSDIQNNKLTWPLIKTFELCSEPDKIKIVKNYGKNNLACVKVIDSL
YEQYKIRKHYESYEKAQKAKILSAINELHHEGIEYVLKYLLEILFTGV

Vector:p15-tev-lic

Growth

Medium:TB
Antibiotics:
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 °C for 3 hours. The culture was then transferred into 1.8 L of above-specified growth medium with Antibiotics and 0.3 mL of antifoam (Sigma) in a 2 L 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 °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. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. Each 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. EDTA was immediately added to the elution fraction to 1 mM; and DTT was added to 1 - 5 mM after approximately 15 more minutes. The sample was loaded onto a Sephadex S200 26/60 gel filtration column pre-equilibrated with 10 mM HEPES, pH 7.5 and 500 mM NaCl. The collected fractions corresponding to the correct eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel. The concentrated sample was stored at 4 °C.

Extraction

Procedure
Cells 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 ºC were thawed overnight at 4 ºC 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 ºC.
Concentration:11.2 mg/mL
Ligand
MassSpec:
Crystallization:1.5 microL of protein at 11.2 mg/mL containing an additional 10 mM ZOL, 10 mM IPP and 10 mM MgCl2 was mixed with 1.5 microL of reservoir solution (20% PEG 3350, 200 mM Li2SO4, 100 mM Tris, 8.5 at 18 °C) and incubated in hanging drops over 350 microL of reservoir solution. Crystals appeared overnight.
NMR Spectroscopy:
Data Collection:
Data Processing: