Pv-2CysPrx

PDB:2I81

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession: Pv118545
Entry Clone Source: Plasmodium vivax Salvador I genomic DNA
SGC Clone Accession:
Tag:N-terminal: His6-tag with integrated thrombin protease site: mgsshhhhhhssglvpr*gs
Host:E. coli BL21-(DE3)-Rosetta Oxford (customized strain)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsPTYVGKEAPFFKAEAVFGDNSFGEVNLTQFIGKKYVLLYFYPLDFTFVCPSEIIALDKALDAFHERNV
ELLGCSVDSKYTHLAWKKTPLAKGGIGNIKHTLLSDITKSISKDYNVLFDDSVSLRAFVLIDMNGIVQHLLVNNLAIGRSVDEILRI
IDAIQHHEKYGDVCPANWQKGKVSMKPSEEGVAQYLSTL

Vector:p28a-thrombin-LIC

Growth

Medium:
Antibiotics:
Procedure: Pv-2CysPrx was expressed in E. coli BL21-(DE3)-Rosetta Oxford 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 ºC. The culture was transferred into 50 mL of TB with 50 microgram/mL kanamycin in a 250 mL shaking flask and incubated at 37 ºC 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 of ~5, cooled to 15 ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

Purification

Procedure

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 (50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, and 5 % glycerol) 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:8 mg/mL for Pv-2CysPrx with His6-tag.
Ligand
MassSpec:
Crystallization: Purified Pv-2CysPrx was crystallized using the hanging drop vapor diffusion method in a VDXm plate with 350 µL of mother liquor at 18 ºC. 1.5 µL of the protein solution treated with 5 mM TCEP was mixed with 1.5 µL of the reservoir solution containing 19% PEG 3350, 150 mM Li3Citrate. Crystals appeared overnight.
NMR Spectroscopy:
Data Collection:
Data Processing: