

Pf-OMPDC

PDB:2Q8L

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:PF10_0225

Entry Clone Source:*Plasmodium falciparum* 3D7 genomic DNA

SGC Clone Accession:PF10_0225::D8 from plate 2000

Tag:N-terminal His-tag with integrated Thrombin protease site: mgsshhhhhssglvprgs

Host:BL21 (DE3) CodonPlus-RIL from Strategene

Construct

Prelude:

Sequence:

mgsshhhhhssglvprgsMGFKVKLEKRRNAINTCLCIGLDPDEKDIEFMKNEKENNYNNIKKNLKEKYINNVSIIKKDILLKAPD
NIIREEKSEEFFYFFNHFCFYIINETNKYALTFKMNAFYIPYGSVGIDVLKNVFDYLYELNIPTILDMKINDIGNTVKNYRKFFIE
YLKSDSCTVNIYMGTNMLKDICYDEEKNKYSAFVLVKTTPDSAIFQKNLSLDNKQAYVIMAQEALNMSSYLNLEQNNEFIGFVVG
ANSYDEMNYIRTYFPNCYILSPGIGAQNGLHKTLTNGYHKSYEKILINIGRAITKNPYPQKAAQMYDQINAILKQNMES

Vector:pET28A-LIC

Growth

Medium:Terrific Broth (TB)

Antibiotics:50 microG/mL kanamycin and 25 microG/mL chloramphenicol

Procedure:Terrific Broth (TB)50 µg/mL kanamycin and 25 µg/mL chloramphenicolA 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 2L 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 cell lysate was loaded onto a column containing 10 g DE-52 resin from Whatman (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer) and then directly onto a 3 mL Ni-NTA (Qiagen) column. When all the lysate was loaded, the two column system was washed with 20 mL binding buffer. The Ni-NTA column was then washed with 200 mL of

Wash Buffer. After washing, the protein was eluted from the Ni-NTA column with 15-20 mL of Elution Buffer. EDTA was added immediately to 1 mM; and DTT was added to 1 mM 15 minutes later.

The protein was put in a dialysis cassette (Pierce) for overnight dialysis in Crystal Buffer. The following day the protein was concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). Finally aliquots of the purified PY02252 protein were labeled and stored at -80°C.

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 °C were thawed overnight at 4 °C 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 ~75,000 x g for 20 minutes at 10 °C.

Concentration:

Ligand

MassSpec:

Crystallization: The protein was crystallized by means of sitting drop vapor diffusion in a 96-well Intelli-Plate. The plate was set with 1.0 µL protein and 1.0 µL buffer in each drop, and 100 µL reservoir volume per well. Crystals grew overnight in 15% PEG 10000, pH 6.5 at 20 °C.

NMR Spectroscopy:

Data Collection:

Data Processing: