Pv-OMPDC-6-aza-UMP

PDB:2GUU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession: Pv111555
Entry Clone Source: Plasmodium vivax Salvador I gDNA (kindly donated by John Barnwell of CDC)
SGC Clone Accession:PV-PF10_0225:; plate G:D12
Tag:N-terminal His-tag with integrated Thrombin protease site: mgsshhhhhhssglvprgs
Host:BL21 (DE3) CodonPlus-RIL from Strategene

Construct

Prelude:
Sequence:

```
mgsshhhhhhssglvprgsNLKIKLQKRRDEVNTCLCIGLDPDEADIKSFMQSEKQNGYQSVKKNLSNSGSSSSSSNSSSGKGELFA
PQMGGQMLLAETPPKEAQEKDEFFYFFNHFCFYIINETKEYALAYKMNFAFYLPYGSLGVDVLKNVFDYLHHLNVPTILDIKMNDIG
NTVKHYRKFIFDYLRSDSCTANIYMGTQMLRDICLDEECKRYYSTFVLVKTTNADSHIFQNRLSLDGKEAYVVIAEEAQKMAKQLHL
EENGEFVGFVVGANCYDEIKKIRELFPDCYILAPGVGAQKGDLRKMLCNGYSKNYEKVLINVGRAITKSGSPQQAAREYHQQIKEVL
AELQE
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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 ºC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 ºC 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 ºC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

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 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 18,000 psi. The cell lysate was centrifuged at ~75,000 x g for 20 minutes at 10 ºC.
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 microL protein and 1.0 microL buffer in each drop, and 100 microL reservoir volume per well. Crystals grew overnight in 30% PEG 4K, 0.2M Sodium acetate, 0.1M Tris HCl, pH 8.5 at 18 ºC.
NMR Spectroscopy:
Data Collection:
Data Processing: