

Pk-TCTP: Plasmodium knowlesi TCTP (ortholog of PFE0545c)

PDB:1TXJ

Revision

Revision Type:created

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Revision Date:created

Entry Clone Accession:gi:62901132

Entry Clone Source:Plasmodium knowlesi H genomic DNA

SGC Clone Accession:PKN-PFE0545c;; plate MAC008:B10

Tag:N-terminal: His-tag with integrated TEV protease site: mgsshhhhhssgrenlyfqghm. C-terminal: gs

Host:BL21 (DE3) Rosetta 2

Construct

Prelude:

Sequence:

mgsshhhhhssgrenlyfqghmKVYKDVFTNDEVCSDSYNQEDPFGIADFREIAFEVKSNKRIKGNDYGIADNSEEAVDGMGADV
EQVIDIVDSFQLTSTSLSKKEYSVYIKNYMQKILKYLEEKKPDRVDVFKTKAQPLIKHILTNFDDFEFYMGESLMDAGLTYSYYKG
EEVTPRFVYISDGLYEEKYgs

Vector:p11

Growth

Medium:Studier auto-induction media

Antibiotics:50 microG/mL carbenicillin

Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of above-specified medium with Antibiotics 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 in a 2L baffled flask and grown at 37 degC overnight in an Innova shaker from New Brunswick Scientific (150 rpm).

Purification

Procedure

The cleared lysate was loaded onto a column of 3 mL Ni-NTA from Qiagen at 4 degC. The column was washed with 150 mL Wash Buffer and the protein was eluted with 15 mL Elution Buffer. About 10 mg of pure protein was obtained from 1L of cell culture. The purified protein

was dialyzed overnight into Crystal Buffer at 4 degC and concentrated using a Amicon Ultra centrifugal filter device from Millipore (15 kD cutoff).

Extraction

Procedure

Cells were centrifuged and the cell pellets were resuspended in binding buffer with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication (1x 30 sec). Lysate was cleared by centrifugation at 35,000 x g and passed through DE52 from Whatman in 0.5 M NaCl.

Concentration: 20 mg/mL

Ligand

MassSpec:

Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a VDXm plate. The plate was set with 1.5 microL uncleaved protein (20 mg/mL) and 1.5 microL buffer in each drop, and 350 microL reservoir volume per well. Crystals emerged in 1.38 M Na Citrate, 0.1 M Hepes and pH 7.5 at 20 degC.

NMR Spectroscopy:

Data Collection:

Data Processing: