Pf-PCMT

PDB:2PBF

Revision

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Entry Clone Accession:PF14_0309
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SGC Clone Accession:
Tag:
Host:BL21 (DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

gNNMYKLSENNHKSLLENLKRRGIIDDDDVYNTMLQVDRGKYIKEIPYIDTPVYISHGVTISAPHMHALSLKRLINVLKPGSRAIDV
GSGSGYLTVCMAIKMNVLENKNSYVIGLERVKDLVNFSLENIKRDKPELLKIDNFKIIHKNIYQVNEEEKKELGLFDAIHVGASASE
LPEILVDLLAENGKLIIPIEEDYTQVLYEITKKNGKIIKDRLFDVCFVSLKKN

Vector:p15-mhl

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 degC. The culture was transferred into 50 mL of TB 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 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 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure
The cleared lysate was loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) column (pre-equilibrated with Binding Buffer) at approximately 2.0-3.0 mL/min. The Ni-NTA column was then washed with 150 mL of Wash Buffer. After washing, the protein was eluted with Elution Buffer. EDTA was immediately added to the elution fraction to 1 mM; and DTT was added to 5 mM after approximately 15 more minutes. The eluted Pf-PCMT was applied to a Sephadex S200 26/60 gel

filtration column pre-equilibrated with Gel Filtration Buffer. The fractions corresponding to the eluted protein peak were collected.

The His-tag was cleaved with TEV protease for three hours at room temperature in the presence of 1mM DTT. Mass Spectrometry result confirmed his-tag was fully cut. The cleaved sample was applied to a 0.5 ml Ni-NTA column pre-equilibrated with Binding buffer. The flow-through was collected; and the column was rinsed with an additional 5 mL of Binding Buffer. These fractions were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). Pf-PCMT was concentrated and flash frozen and stored at -80degC.

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Bufferwith protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF). Resuspended pellets stored at -80degC were thawed overnight at 4degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pre-treated with 0.5% CHAPS and 500 units of benzonase for 40 minutes at room temperature. After 10 minutes sonication, the cell lysate was centrifuged at ~75000 x g for 20 minutes at 10 degC.
Concentration:25.9 mg/mL
Ligand
MassSpec:
Crystallization: The protein, with S-adenosyl-L-homocysteine (SAH) to 2 mM, was crystallized using the sitting drop vapor diffusion method at 18degC. 0.5 microL of the protein-ligand solution was mixed with 0.5 microL of the reservoir solution containing 25% PEG3350, 0.2 M ammonium sulphate and 0.1 M Hepes pH 7.2. Crystals appeared in 1-2 days.
NMR Spectroscopy:
Data Collection:
Data Processing: