

Pv-MECPs

PDB:3B6N

Revision

Revision Type:created

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Entry Clone Accession:Pv089580

Entry Clone Source:

SGC Clone Accession:Pv003920:Y102-S287:B10

Tag:mgsshhhhhhsgrenlyfq

Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:

Sequence:

gYDGVRIGQGYDIHQIRVGPPEDIVADTTADTAANTADPNKQSFKRLTIGGPVETISVLSHSDGDVIFHALVDALLGGMCSSDLGT
LFPDGSPKYKNKNSLSFLRYARLLYKRNYAIANVIIIAEVPKISPIREEIVRNIISSALGISESQVSLKGKTHEQLGPVGQKKAI
ECFANALLIRKQS

Vector:p15-tev-lic

Growth

Medium:TB

Antibiotics:

Procedure:Pv003920 was expressed in E. coli BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with 50 microg/mL ampicillin in a 250 mL shaking flask and incubated at 37 degC for 3 hours. Then the culture was transferred into 1.8 L of TB with 50 microg/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD 600 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 column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and

subsequently onto a 1.0 Å 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Å 1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 Å 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. TCEP was added to 1 Å 5 mM after approximately 15 more minutes.

The His6-tag was cleaved with TEV overnight at 4 degC in the presence of 1 mM TCEP and dialysed into Crystal Buffer (10 mM HEPES, pH 7.5, 500 mM NaCl). The cleaved sample was loaded onto a Sephadex S75 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak eluting at 218mL corresponding to trimeric protein were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evaluated by mass spectroscopy and SDS-PAGE gel. The concentrated protein was stored at 4 degC.

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 with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 degC were thawed overnight at 4 degC 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 rpm) for 20 minutes at 10 degC.

Concentration:

Ligand

MassSpec:

Crystallization: MAY5CA:D1-12 The protein was crystallized at 20 degC in using the Sitting Drop Vapor Diffusion method.

NMR Spectroscopy:

Data Collection:

Data Processing: