Pf-SRM + dcAdoMet

PDB:2PT9

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PF11_0301
Entry Clone Source: Codon optimized synthetic DNA
SGC Clone Accession:
Tag:N-terminal: His6-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-R3

Construct

Prelude:
Sequence:

gSKKWFSEFSIMWPGQAFSLKIKKILYETKSKYQNVLVFESTTYGKVLVLDGVIQLTEKDEFAYHEMMTHVPMTVSKEPKNVLVVGG
GDGGIIRELCKYKSVENIDICEIDETVIEVSKIYFKNISCGYEDKRVNVFIEDASKFLENVTNTYDVIIVDSSDPIGPAETLFNQNF
YEKIYNALKPNGYCVAQCESLWIHVGTIKNMIGYAKKLFKKVEYANISIPTYPCGCIGILCCSKTDTGLTKPNKKLESKEFADLKYY
NYENHSAAFKLPAFLLKEIENI

Vector:p15-Tev-LIC

Growth

Medium:TB
Antibiotics:
Procedure: Pf-SRM was expressed in E. coli BL21-(DE3)-Rosetta Oxford in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively). A single colony was inoculated into 100mL of LB with of ampicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively) in a 250 mL baffled flask and incubated with shaking at 250 rpm overnight at 37 ºC. The culture was transferred into 1.8 L of TB with ampicillin/chloramphenicol (100 µg/mL and 34 µg/mL respectively) and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of 4.5, cooled to 15 ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

Purification

Procedure
The cleared 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). The DE52 column was further washed with 20 mL of Binding Buffer.

The lysate was subsequently loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) column (pre-equilibrated with Binding Buffer) at approximately 1-1.5 mL/min. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with 15 mL of 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-SRM was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with 10 mM HEPES, pH 7.5 and 500 mM NaCl. The fractions corresponding to the eluted protein peak were collected.
The His-tag was cleaved with TEV protease overnight at 4 ºC in the presence of 1mM DTT. The cleaved sample was applied to a 1mL 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). Using the same device, Binding Buffer was exchanged to Crystal Buffer by adding 15 mL Crystal Buffer twice to the portion of protein solution that was concentrated to around 0.5 mL.Finally, the protein was concentrated and flash frozen and stored at -80 ºC.

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 ºC 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 16000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at 24000 rpms (~75000 x g) for 20 minutes at 10 ºC.
Concentration:14.2 mg/mL for Pf-SRM (construct ID: PF11_0301:s:S40-I321:E7, without histag).
Ligand
MassSpec:
Crystallization: Purified Pf-SRM was crystallized using the sitting drop vapor diffusion method at 18 ºC in 25% PEG3350, 0.1 M Ammonium Sulphate and 0.1 M Bis-Tris pH 5.5 and 2.5 mM dcAdoMet.
NMR Spectroscopy:
Data Collection:
Data Processing: