ISPD

PDB:4CVH

Revision

Revision Type:created
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Entry Clone Accession:NM_001101426.1
Entry Clone Source: Collaborator
SGC Clone Accession:
Tag:N-terminal, TEV protease cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

MGHHHHHHSSGVDLGTENLYFQSMHPQAVAAVLPAGGCGERMGVPTPKQFCPILERPLISYTLQALERVCWIKDIVVAVTGENMEVM
KSIIQKYQHKRISLVEAGVTRHRSIFNGLKALAEDQINSKLSKPEVVIIHDAVRPFVEEGVLLKVVTAAKEHGAAGAIRPLVSTVVS
PSADGCLDYSLERARHRASEMPQAFLFDVIYEAYQQCSDYDLEFGTECLQLALKYCCTKAKLVEGSPDLWKVTYKRDLYAAESIIKE
RISQEICVVMDTEEDNKHVGHLLEEVLKSELNHVKVTSEALGHAGRHLQQIILDQCYNFVCVNVTTSDFQETQKLLSMLEESSLCIL
YPVVVVSVHFLDFKLVPPSQKMENLMQIREFAKEVKERNILLYGLLISYPQDDQKLQESLRQGAIIIASLIKERNSGLIGQLLIA

MHHHHHHSSGVDLGTENLYFQ*SM is the purification tag plus TEV protease recognition site *.
Vector:pFB-LIC-Bse Baculovirus transfer vector (Bac-to-bac)

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Binding/Lysis Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 20 mM Imidazole pH 7.5, 0.5 mM TCEP Wash Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.5, 0.5mM TCEP Elution Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.5, 0.5mM TCEPGel Filtration Buffer: 10 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 0.5mM TCEP
Procedure
Cell Lysis

Cell pellets were resuspended in 50 mL lysis buffer. The cells were lysed by sonication for 7.5 min 5sec on/ 5 sec off. The cell debris were pelleted at 35 000 g and the supernatant used for further purification.

Column 1
Ni-NTA (1.25 ml volume in a gravity-flow column).
The clarified cell extract was incubated with 5.0 ml pre-equilibriated 50%. Ni_NTA bead suspension for 1 hour at 4°C with rotation. The suspension was centrifuged at 900g for 5 min. The supernatant was poured away and the beads were resuspended in lysis buffer and transferred onto a gravity column. The column was then washed with 30ml Binding Buffer (2 x 15ml) and 50 ml Wash Buffer (2 x15 ml). The protein was eluted with 25 ml of elution Buffer in 5 x 2.5 ml fractions.

Column 2
Superdex s200 16/60 Gel Filtration.
Elution fraction 1&2 were concentrated to 5 mL and applied directly to the size exclusion chromatography column, s200 (pre-equilibriated in GF buffer).

Enzymatic treatment and purification
The N-terminal His6- tag was cleaved by incubating overnight with TEV (20°C). Cleaved protein was purified by batch binding on 125uL pre-equilibriated 50% Ni-NTA bead solution. The column was then washed with 2x500uL Gel Filtration buffer,2x500uL Binding buffer, 2x500uLWash buffer, and finally 2x500uL of Elution buffer.

Extraction

Buffers
Procedure
Expression strain

Expression system: BEVS
Host cell line: Sf9
Expression medium: Insect-Xpress
Cell density at the time of infection: 2 x 10E6 cells/ml
Expression temperature: 27°C
Virus: used 3 ml of P2/L (unless stated otherwise)
Expression time point: ~ 70-72 hrs
Culture vessel: 3L glass flasks without baffles
Culture volume: Each 3 L flask contained 1L
Total volume 6L

Cell harvest
Cells were harvested by centrifugation at 900g after which the media were poured out and the cell pellet placed in a -80°C freezer.
Concentration: To set up plates the sample was concentrated to 14.76 mg/ml using a 10 kDa mwco concentrator.
Ligand
MassSpec: Expected mass: 46049.6 Da
Measured mass: 46048.96 Da
Crystallization: Crystals were grown by vapour diffusion in sitting drop at 20°C. A sitting drop consisting of 50 nl protein and 100nl well solution was equilibrated against well solution containing 0.1M bis-tris pH 6.5, 25%(w/v) PEG 3350.

NMR Spectroscopy:
Data Collection: Resolution: 2.4 Å
X-ray source: Diamond Light Source beamline I03
Data Processing: