AASDHPPT

PDB:2BYD

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:AASDHPPTA-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag: His 6 with Tev cleavage site: mgsshhhhhhhssgrenlyfqgh
Host:E.coli BL21 DE3-R3

Construct

Prelude:
Sequence:

mgsshhhhhhhssgrenlyfqghMEGVRWAFSCGTWLPSRAEWLLAVRSIQPEEKERIGQFVFARDAKAAMAGRLMIRKLVAEKLNI
PWNHIRLQRTAKGKPVLAKDSSNPYPNFNFNISHQGDYAVLAAEPELQVGIDIMKTSFPGRGSIPEFFHIMKRKFTNKEWETIRSFK
DEWTQLDMFYRNWALKESFIKAIGVGLGFELQRLEFDLSPLNLDIGQVYKETRLFLDGEEEKEWAFEESKIDEHHFVAVALRKPDGS
RHQDVPSQDDSKPTQRQFTILNFNDLMSSAVPMTPEDPSFWDCFCFTEEIPIRNGTKS

Vector:pCOEX-1

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Column 1 : Ni-affinity, HisTrap, 1 mL (GE/Amersham)

Buffers: Lysis buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP. Wash buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 50 mM imidazole, 0.5 mM TCEP. Elution buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP.

Procedure: The cell extract was loaded on the column at 0.8 mL/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of Lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 mL/min. The eluted peak of A280 was automatically collected.

Column 2 : Gel filtration, Hiload 16/60 Superdex 200 prep grade 120 mL (GE Healthcare)

Buffers: GF buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.

Extraction

Procedure
Lysis buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, Complete® protease inhibitors (1 tablet/50 mL).

Frozen cell pellets were thawed on ice over night and resuspended in a total volume of 40 mL lysis buffer, the cells were disrupted by high pressure (20 psi) followed by sonication.

Nucleic acids and cell debris were removed by adding 0.15% PEI from a 5% (w/v) stock, stirring for 15 minutes, then centrifugation for 20 minutes at 40,000xg. The supernatant was then further clarified by filtration (0.45 µm).
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the following conditions: Vapour diffusion method, sitting drop, 293K, 0.05 M H3Cit/Na3Cit, pH 5.7, 14% PEG 3350.
NMR Spectroscopy:
Data Collection: Resolution: 2.2Å, X-ray source: Synchrotron SLS -X10, multiple wavelength.
Data Processing: