FKBP2

PDB:2PBC

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_004461
Entry Clone Source:MGC
SGC Clone Accession:fkbp02.043.142; SDC065:B03
Tag:mgsshhhhhhssglvprgs
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsPIKSRKGDVLHMHYTGKLEDGTEFDSSLPQNQPFVFSLGTGQVIKGWDQGLLGMCEGEKRKLVIPSEL
GYGERGAPPKIPGGATLVFEVELLKIERRTEL

Vector:p28a-thrombin-lic

Growth

Medium: Terrific Broth (TB)
Antibiotics:
Procedure:FKBP02 was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37 ºC to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15 ºC. The culture was centrifuged and the cell pellets were collected and stored at -80 ºC.

Purification

Procedure
Clarified lysate is diluted 1:2 in lysis buffer, and 3mL of His- Ni-NTA resin (Qiagen 30450) is added to the lysate. This mixture is rocked at low velocity for at least 20 minutes at 4oC. The lysate is then spun at 500xg for 5 minutes, and the supernatant is decanted. The pelleted beads are mixed with 20 mL lysis buffer, and then the beads are transferred to a gravity column, followed by washing with 5 column volumes of low imidazole buffer at approximately 3 mL/min. Samples are eluted from the Ni-NTA resin by exposure to 10 mL IMAC elution buffer at 1mL/min flow rate. 1unit of thrombin (Sigma T9681) per milligram of protein is added to the IMAC eluate and stored without shaking, overnight, at 4oC. An XK 16x65 column (part numbers 18-1031-47 and

18-6488-01, GE Healthcare) packed with HighLoad Superdex 200 resin (10-1043-04, GE Healthcare) is pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTAxpress (18-6645-05, GE Healthcare) at a flow rate of 3 mL/min. 10 mL of sample is loaded onto the column at 1.5 mL/min, and 2mL fractions are collected into 96-well plates (VWR 40002-012) using peak fractionation protocols). Peak fractions are analyzed for purity using SDS-PAGE or visual analysis of the chromatogram and pooled. Purified proteins are concentrated using 15 mL concentrators with 10,000 MWCO (Amicon, UFC901024) to a final concentration of 20 mg/mL for crystallographic screening.

Extraction

Procedure
Cell pellets contained in bags (Beckman 369256) obtained from 4L culture are thawed by soaking in warm water. Each cell pellet is resuspended in 20 mL lysis buffer and then homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis is accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol is 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 6 minutes total sonication time per pellet. The resulting lysate was then centrifuged at 63 000 xg for 30 minutes at 10 ºC.
Concentration:20 mg/mL
Ligand
MassSpec:
Crystallization:32% PEG MME550, vapor diffusion, 1µL+1µL hanging drop, temperature 298.0K, crypprotected in paratone-N
NMR Spectroscopy:
Data Collection:
Data Processing: