SLK

PDB:4USF

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:SGC Oxford
SGC Clone Accession:
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

SMKQYEHVTRDLNPEDFWEIIGELGDGAFGKVYKAQNKETSVLAAAKVIDTKSEEELEDYMVEIDILASCDHPNIVKLLDAFYYENN
LWILIEFCAGGAVDAVMLELERPLTESQIQVVCKQTLDALNYLHDNKIIHRDLKAGNILFTLDGDIKLADFGVSAKNTRTIQRRDSF
IGTPYWMAPEVVMCETSKDRPYDYKADVWSLGITLIEMAEIEPPHHELNPMRVLLKIAKSEPPTLAQPSRWSSNFKDFLKKCLEKNV
DARWTTSQLLQHPFVTVDSNKPIRELIAEAKAEVTEEVEDGKE The N-terminal residues, SM, derive from th
e vector.

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure
Cell Lysis: The resuspended cell pellet was thawed and lysed by sonication. PEI (polyethyleneimine) was added to a final concentration of 0.15 %. The cell debris and precipitated DNA were spun down.Lysis Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP. Purification: Column 1: 5 mL of Ni-Sepharose in a 2.5 cm diameter gravity flow column. Column 1 Buffers: Binding Buffer: 50 mM Hepes pH 7.4, 500 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP. Wash Buffer 1: As Binding Buffer except 40 mM imidazole and 1M NaCl.Wash Buffer 2: As Binding Buffer except 60 mM imidazole. Elution Buffer: As Binding Buffer except 250 mM imidazole. Column 1 Procedure: The clarified supernatant was passed through the column. The column was washed with 50 mL of Binding Buffer and 50 mL

each of Wash Buffer 1 and 2. 25 ml of Elute Buffer was passed through to elute the protein. TEV protease was added and the sample was dialysed into GF Buffer overnight. GF Buffer: 50 mM Hepes pH 7.5, 300 mM NaCl, 0.5 mM TCEP Column 2: Ni-Sepharose. The digested sample was passed through the resin, which was eluted with GF Buffer containing 10mM, 20mM, 30mM and then 40mM imidazole. Column 3: S200 16/60 Gel Filtration (GE Healthcare) Column 3 Buffers: 50 mM HEPES, 300 mM NaCl, 5 mM DTT Column 3 Procedure: The eluted protein was concentrated to 5 ml volume and injected onto the column.

Extraction

Buffers
Procedure
Expression strain: BL-21(DE3)-R3-lambda-ppase (coexpresses lambda phosphatase). Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure. Expression: Colonies were used to inoculate 50 mL of LB media containing 50 µg/mL kanamycin and 34 µg/mL chloramphenicol, which was placed in a 37°C shaker overnight. The next day 4x 10 mL of this starter culture was used to inoculate 4x 1L of LB media containing 37.5 µg/mL kanamycin in 2L baffled shaker flasks. When the OD600 was approximately 0.45, the temperature was reduced to 20°C and when the OD600 was approximately 0.6 the cells were induced by the addition of 0.5 mM IPTG. The expression was continued overnight. Cell harvest: Cells were spun at 5000rpm for 10 mins and the pellets resuspended in Lysis Buffer and then frozen at -20°C.
Concentration: The protein was concentrated to 13 mg/ml (measured by 280 nm absorbance).
Ligand
MassSpec:
Crystallization: Compound SB-440719 was added to 1 mM. Crystals grew from a 1:2 ratio of protein and precipitant solution (20% PEG6000, 10% ethylene glycol, 0.1M MES pH 6.0, 0.1M magnesium chloride), using the vapour diffusion method. Crystals were cryo-protected by equilibration into precipitant solution containing 25% ethylene glycol, and then flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: