STK10

PDB:4USE

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:

SMRKSREYEHVRRDLDPNEVWEIVGELGDGAFGKVYKAKNKETGALAAAKVIETKSEEELEDYIVEIEILATCDHPYIVKLLGAYYH
DGKLWIMIEFCPGGAVDAIMLELDRGLTEPQIQVVCRQMLEALNFLHSKRIIHRDLKAGNVLMTLEGDIRLADFGVSAKNLKTLQKR
DSFIGTPYWMAPEVVMCETMKDTPYDYKADIWSLGITLIEMAQIEPPHHELNPMRVLLKIAKSDPPTLLTPSKWSVEFRDFLKIALD
KNPETRPSAAQLLEHPFVSSITSNKALRELVAEAKAEVMEE The N-terminal residues, SM, derive from the
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, 200 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, 200 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP. Wash Buffer 1: As Binding Buffer except 1 M NaCl and 40 mM imidazole.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 100 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 left overnight. Column 2: S200 16/60 Gel Filtration (GE Healthcare) Column 2 Buffers: GF Buffer: 50 mM Hepes pH 7.5, 300 mM NaCl, 0.5 mM TCEP Column 2 Procedure: The eluted protein was concentrated to 5 ml volume and injected onto the column. Column 3: Ni-Sepharose. The pooled fractions from gel filtration were passed through the resin.

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 70 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 6x 10 mL of this starter culture was used to inoculate 6x 1L of LB media containing 35 µg/mL kanamycin in 2L baffled shaker flasks. When the OD600 was approximately 0.3, 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 6000x g for 10 mins and the pellets resuspended in Lysis Buffer and then frozen at -20°C.
Concentration:
Ligand
MassSpec: Expected Observed34251.4 34254.0
Crystallization: Compound SB-633825 was added. The protein was concentrated to 53.5 mg/ml (measured by 280 nm absorbance), although this measurement was likely influenced by the compound. Crystals grew from a 1:2 ratio of protein and precipitant solution (50%(v/v) PEG 300, 0.1M cacodylate pH 6.5, 0.2M Magnesium Chloride), using the vapour diffusion method.
NMR Spectroscopy:
Data Collection:
Data Processing: