STK-10 (4AOT) Materials & Methods

Entry Clone Source: SGC Toronto

Vector: pNIC-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Amplified construct sequence:

ATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACC
TGTACTTCCAATCCATGAGAAAGTCC
CGCGAATATGAGCACGTCCGCCGCGA
CCTGGACCCCAACGAGGTGTGGGAGA
TCGTGGGCGAGCTGGGCGACGGCGCC
TTCGGCAAGGTTTACAAGGCCAAGAA
TAAGGAGACGGGTGCTTTGGCTGCGG
CCAAAGTCATTGAAACCAAGAGTGAG
GAGGAGCTGGAGGACTACATCGTGGA
GATTGAGATCCTGGCCACCTGCGACC
ACCCCTACATTGTGAAGCTCCTGGGA
GCCTACTATCACGACGGGAAGCTGTG
GATCATGATTGAGTTCTGTCCAGGGG
GAGCCGTGGACGCCATCATGCTGGAG
CTGGACAGAGGCCTCACGGAGCCCCA
GATACAGGTGGTTTGCCGCCAGATGC
TAGAAGCCCTCAACTTCCTGCACAGC
AAGAGGATCATCCACCGAGATCTGAA
AGCTGGCAACGTGCTGATGACCCTCG
AGGGAGACATCAGGCTGGCTGACTTT
GGTGTGTCTGCCAAGAATCTGAAGAC
TCTACAGAAACGAGATTCCTTCATCG
GCACGCCTTACTGGATGGCCCCCGAG
GTGGTCATGTGTGAGACCATGAAAGA
CACGCCCTACGACTACAAAGCCGACA
TCTGGTCCCTGGGCATCACGCTGATT
GAGATGGCCCAGATCGAGCCGCCACA
CCACGAGCTCAACCCCATGCGGGTCC
TGCTAAAGATCGCCAAGTCAGACCCT
CCCACGCTGCTCACGCCCTCCAAGTG
GTCTGTAGAGTTCCGTGACTTCCTGA
AGATAGCCCTGGATAAGAACCCAGAA
ACCCGACCCAGTGCCGCGCAGCTGCT
GGAGCATCCCTTCGTCAGCAGCATCA
CCAGTAACAAGGCTCTGCGGGAGCTG
GTGGCTGAGGCCAAGGCCGAGGTGAT
GGAAGAGTGA

Purified protein sequence (Tag sequence in lowercase):

smRKSREYEHVRRDLDPNEVWEIVGE
LGDGAFGKVYKAKNKETGALAAAKVI
ETKSEEELEDYIVEIEILATCDHPYI
VKLLGAYYHDGKLWIMIEFCPGGAVD
AIMLELDRGLTEPQIQVVCRQMLEAL

NFLHSKRIIHRDLKAGNVLMTLEGDI
RLADFGVSAKNLKTLQKRDSFIGTPY
WMAPEVVMCETMKDTPYDYKADIWSL
GITLIEMAQIEPPHHELNPMRVLLKI
AKSDPPTLLTPSKWSVEFRDFLKIAL
DKNPETRPSAAQLLEHPFVSSITSNK
ALRELVAEAKAEVMEE

Tags and additions: Cleavable N-terminal His6 tag

Host: BL21(DE3)-R3-pRARE2

Growth Medium & Induction Protocol: Colonies were used to inoculate 50 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol in a 250 ml baffled shaker flask, 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 35 µg/ml kanamycin in 2L baffled shaker flasks. When the OD_{600} was approximately 0.45, the temperature was reduced to 20°C and when the OD_{600} was approximately 0.6 the cells were induced by the addition of 0.5 mM IPTG. The expression was continued overnight. Cells were spun at 5000rpm for 10 mins and the pellets resuspended in Lysis Buffer and then frozen at -20°C.

Cell lysis and purification: 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, 0.2 mM PMSF

Column 1: 5 ml of Ni-Sepharose in a 2 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.

Column 2: S200 16/60 Gel Filtration (GE Healthcare)

Column 2 Buffers:
Gel Filtration buffer: 25 mM Hepes pH 7.4, 300 mM NaCl, 0.5 mM TCEP

Column 2 Procedure: The eluted protein was concentrated to 5 ml volume and injected onto the column.

TEV Protease digestion: The fractions containing STK10A were pooled and TEV protease was added. The sample was left at 4°C overnight. The cleaved sample was passed through Ni-Sepharose

Concentration: The STK10 was concentrated to 13.6 mg/ml (measured by 280 nm absorbance).

Crystallization: Compound GW830263A was added to STK10 protein to a concentration of 1 mM. Crystals grew from a 1:2 ratio of protein and precipitant solution (0.2M NaI, 0.1M

BisTrisPropane pH 6.5, 20% PEG 3350, 10.0% Ethylene Glycol), using the vapour diffusion method.

Data Collection:
Resolution: 1.49Å **X-ray source**: Diamond IO4-1
X-ray source: Diffraction data were collected from a single crystal Diamond beamline I04 at a single wavelength of 0.9763Å and the structure was refined to 1.49Å.
Data collection: Crystals were cryo-protected by equilibration into precipitant solution containing 25% ethylene glycol, and then flash frozen in liquid nitrogen. Data was collected at Diamond, beamline I04-1.