YANK1A (4FR4) Materials & Methods

Entry Clone Source: Synthetic DNA

SGC Construct ID: YANK1A-c051

Vector: pFB-LIC-Bse. Details [PDF]; Sequence [FASTA] or [GenBank].

DNA sequence:

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ATGGGCCACCATCATCATCATCATTC
TTCTGGTGTAGATCTGGGTACCGAGA
ACCTGTACTTCCAATCCATGCCACCA
GTGTTTGATGAAAATGAAGATGTCAA
CTTTGACCACTTTGAAATTTTGCGAG
CCATTGGGAAAGGCAGTTTTGGGAAG
GTCTGCATTGTACAGAAGAATGATAC
CAAGAAGATGTACGCAATGAAGTACA
TGAATAAACAAAAGTGCGTGGAGCGC
AATGAAGTGAGAAATGTCTTCAAGGA
ACTCCAGATCATGCAGGGTCTGGAGC
ACCCTTTCCTGGTTAATTTGTGGTAT
TCCTTCCAAGATGAGGAAGACATGTT
CATGGTGGTGGACCTCCTGCTGGGTG
GAGACCTGCGTTATCACCTGCAACAG
AACGTCCACTTCAAGGAAGAAACAGT
GAAGCTCTTCATCTGTGAGCTGGTCA
TGGCCCTGGACTACCTGCAGAACCAG
CGCATCATTCACAGGGATATGAAGCC
TGACAATATTTTACTTGACGAACATG
GGCACGTGCACATCACAGATTTCAAC
ATTGCTGCGATGCTGCCCAGGGAGAC
ACAGATTACCACCATGGCTGGCACCA
AGCCTTACATGGCACCTGAGATGTTC
AGCTCCAGAAAAGGAGCAGGCTATTC
CTTTGCTGTTGACTGGTGGTCCCTGG
GAGTGACGGCATATGAACTGCTGAGA
GGCCGGAGACCGTATCATATTCGCTC
CAGTACTTCCAGCAAGGAAATTGTAC
ACACGTTTGAGACGACTGTTGTAACT
TACCCTTCTGCCTGGTCACAGGAAAT
GGTGTCACTTCTTAAAAAGCTACTCG
AACCTAATCCAGACCAACGATTTTCT
CAGTTATCTGATGTCCAGAACTTCCC
GTATATGAATGATATAAACTGGGATG
CAGTTTTTCAGAAGAGGCTCATTCCA
GGTTTCATTCCTAATAAAGGCAGGCT
GAATTGTGATCCTACCTTTGAACTTG
AGGAAATGATTTTGGAGTCCAAACCT
CTACATAAGAAAAAAAAGCGTCTGGC
AAAGAAGGAGAAGGATATGAGGAAAT
GCGATTCTTCTCAGACATGTCTTCTT
CAAGAGCACCTTGACTCTGTCCAGAA
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GGAGTTCATAATTTTCAACAGAGAAA
AAGTAAACAGGGACTTTAACAAAAGA
CAACCAAATCTAGCCTTGGAACAAAC
CAAAGACCCACAAGGTGAGGATTGA

Final protein sequence (Tag sequence in lowercase):
mghhhhhhssgvdlgtenlyfq^smP
PVFDENEDVNFDHFEILRAIGKGSFG
KVCIVQKNDTKKMYAMKYMNKQKCVE
RNEVRNVFKELQIMQGLEHPFLVNLW
YSFQDEEDMFMVVDLLLGGDLRYHLQ
QNVHFKEETVKLFICELVMALDYLQN
QRIIHRDMKPDNILLDEHGHVHITDF
NIAAMLPRETQITTMAGTKPYMAPEM
FSSRKGAGYSFAVDWWSLGVTAYELL
RGRRPYHIRSSTSSKEIVHTFETTVV
TYPSAWSQEMVSLLKKLLEPNPDQRF
SQLSDVQNFPYMNDINWDAVFQKRLI
PGFIPNKGRLNCDPTFELEEMILESK
PLHKKKKRLAKKEKDMRKCDSSQTCL
LQEHLDSVQKEFIIFNREKVNRDFNK
RQPNLALEQTKDPQGED

(Pro9 to Asp390)

^ TEV protease recognition site

Tags and additions: Cleavable N-terminal His6 tag

Expression: The YANK1 protein was expressed using baculovirus infected Sf9 cells. Cells were infected at a density of 2,000,000 cells/ml for 48h.

Cell Harvest: Cells were spun at 1000x g for 20 mins and the pellets resuspended in Lysis Buffer and then frozen at -20°C.

Cell lysis: The resuspended cell pellet was thawed and lysed by sonication. PEI (polyethyleneimine) was added to a final concentration of 0.05 %. The cell debris and precipitated DNA were spun down.
Lysis buffer: 50 mM Tris pH 7.8, 200 mM NaCl, 20 mM Imidazole, 0.5 mM TCEP, 1:2000 dilution of Sigma protease inhibitor cocktail.

Column 1: 6 ml of Ni-Sepharose in a 2 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: As Binding Buffer except 1 M NaCl and 40 mM imidazole.
Elution buffer: As Binding Buffer except 60 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 of Wash Buffer 1 and 40 ml of Wash Buffer 2. 36 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: 20 mM Tris pH 7.8, 150 mM NaCl, 0.5 mM TCEP

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

Concentration: The pooled fractions from the the gel filtration were passed through 0.8 ml of Ni-Sepharose (pre-equilibrated into GF Buffer) followed by 5 ml of additional GF Buffer. The resin was eluted with 5 ml of GF Buffer containing 10 mM, 20 mM and then 30 mM imidazole.

The fractions containing YANK1 were combined. Staurosporine was added and the sample concentrated to 27 mg/ml (measured by 280 nm absorbance). The sample was diluted with GF buffer and reconcentrated to reduce the imidazole concentration.

Mass spec characterization:
Expected: 45101.0
Observed: 45101.9

Crystallization: Crystals grew from a 1:2 ratio of protein and precipitant solution (0.2 M Sodium acetate, 20% PEG 3350, 10% Ethylene Glycol), using the vapour diffusion method. (A peptide was added during crystallisation, however the peptide is not visible in the structure and similar crystals also grew in the absence of peptide.)

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 IO4-1.