KCTD13 (potassium channel tetramerization domain containing 13)

PDB Code: 4UIJ

Material and Methods

Entry Clone Source: MGC

Entry Clone Accession: BC036228

SGC Construct ID: KCTD13A-c001

Entry clone accession/ sequence:

ATGTCGGCGGAGGCCTCGGGCCCGGCTGCCGCCGCGGCCCCGTCCCTGGAAGCCCCCAAGCCCT
CGGGTCTCGAGCCT
GGCCCCGCCGCCTACGGTCTCAAGCCGCTGACCCCGAACAGCAAATACGTGAAGCTGAACGTGG
GCGGCTCGTTGCAC
TACACCACGCTGCGCACCCTCACGGGACAGGACACCATGCTCAAAGCCATGTTCAGCGGCCGCG
TGGAGGTGCTGACC
GATGCCGGAGGTTGGGTGCTGATTGACCGGAGCGGCCGTCACTTTGGTACAATCCTCAATTACC
TGCGGGATGGGTCT
GTGCCACTGCCGGAGAGTACGAGAGAACTGGGGGAGCTGCTGGGCGAAGCACGCTACTACCTGG
TGCAGGGCCTGATT
GAGGACTGCCAGCTGGCGCTGCAGCAAAAAAGGGAGACGCTGTCCCCGCTGTGCCTCATCCCCA
TGGTGACATCTCCC
CGGGAGGAGCAGCAGCTCCTGGCCAGCACCTCCAAGCCCGTGGTGAAGCTCCTGCACAACCGCA
GTAACAACAAGTAC
TCCTACACCAGCACTTCAGATGACAACCTACTTAAGAACATCGAGCTGTTCGACAAGCTGGCCC
TGCGCTTCCACGGG
CGGCTACTCTTCCTCAAGGATGTCCTGGGGGGACGAGATCTGTTGCTGGTCTTTCTACGGGCAGG
GCCGCAAAATCGCC
GAGGTGTGCTGCACCTCCATTGTCTATGCTACGGAGAAGAAGCAGACCAAGGTGGAATTTCCAG
AGGCCCGGATCTTC
GAGGAGACCCTGAACATCCTCATCTACGAGACTCCCCGGGGCCCAGACCCAGCCCTCCTGGAGG
CCACAGGGGGAGCA
GCTGGAGCTGGTGGGGCTGGCCGCGGGGAGGATGAAGAGAACCGAGAGCACCGTGTCCGCAGGA
TCCATGTCCGGCGC
CATATCACCCACGACGAGCGTCCTCATGGCCAACAAATTGTCTTCAAGGACTGA

Expressed protein sequence:
MHHHHHHSSGVDLGTENLYFQSMGPA
AYGLKPLTPNSKYVKLNVGGSLHYTT
LRTLTGQDTMLKAMFSGRVEVLTDAG
GWVLIDRSGRHFGTILNYLRDGSVPL
PESTRELGELLGEARYYLVQGLIEDC
QLALQQKRETL

Vector: pNIC28-Bsa4

Tags and additions: MGHHHHHHSSGVDLGTENLYFQ*SM. cleavable N-terminal hexahistidine tag.

Host: BL21(DE3)-R3-pRARE2

Growth medium, induction protocol:

A glycerol stock was used to inoculate a 10 ml starter culture containing LB media and 50 µg/ml kanamycin. The starter culture was grown overnight at 37°C. 1L LB media with 50 µg/ml kanamycin were inoculated with 10 ml of the starter culture. The 1L culture was incubated at 37°C until an OD600nm = 0.4-0.5 was reached. The flasks were then cooled down to 18°C and 0.4 mM IPTG was added to induce protein expression overnight. Cells were harvested by centrifugation at 5000 g at 4°C for 20 min. The cell pellet was resuspended in 15 ml binding buffer (50 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole), transferred to a 50 ml tube, and stored at -20°C.

Extraction buffer, extraction method:

The frozen cells were thawed. The cells were lysed by sonication on ice with the sonicator pulsing ON for 5 sec and OFF for 10 sec. The DNA was precipitated using 0.15% PEI (polyethyleneimine) pH 8. The cell lysate was spun down by centrifugation at 21.5K rpm at 4°C for 1 h. The supernatant was recovered for purification.

Column 1: Ni-Affinity Chromatography. 5 ml of 50 % Ni-sepharose slurry was applied onto a 1.5 x 10 cm column. The column was equilibrated with binding buffer (25ml).

Buffers:

Binding buffer:50 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole
Wash buffer:50 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 30 mM imidazole
Elution buffer:50 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 50 to 250 mM imidazole

Procedure:

The supernatant was applied by gravity flow onto the Ni-sepharose column. The bound protein was eluted by applying a step gradient of imidazole - using 5 ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 mM, 250 mM).

Enzymatic treatment: 0.5mM DTT and 0.4mg of TEV protease was added to the Ni-eluted protein to remove the tag.

Column 2: Size Exclusion Chromatography - S75 HiLoad 16/60 Superdex run on ÄKTA-Express

Buffer:

Gel Filtration buffer: 300 mM NaCl, 50 mM Hepes pH 7.5, 0.5mM TCEP

Procedure: Prior to applying the protein, the S75 16/60 column was washed and equilibrated with gel filtration buffer. The protein was concentrated to 4 ml using an Amicon Ultra-15 filter with a 3 kDa cut-off. The concentrated protein was directly applied onto the equilibrated S75 16/60 column, and run at a flow-rate of 0.4 ml/min. Fractions containing the protein were pooled together.

Column 3: Ni-Affinity Chromatography (reverse). 5 ml of 50 % Ni-sepharose slurry was applied onto a 1.5 x 10 cm column. The column was equilibrated with gel filtration buffer (25ml).

Procedure: Pooled fractions containing cleaved and uncleaved protein from gel filtration were combined and passed over Ni column. 5mM DTT and 5% glycerol was added to the tag-cleaved non-binding fraction and concentrated to 2.75 mg/ml in an amicon 3kD cutoff spin concentrator.

Mass spec characterization: The purified protein had an experimental mass of 13229.6 (after TEV cleavage), that is within the error of the expected mass of 13229.2 Da. The construct was verified correct by DNA sequencing. Mass was determined by LC-MS, using an Agilent LC/MSD TOF system with reversed-phase HPLC coupled to electrospray ionisation and an orthogonal time-of-flight mass analyser. Proteins were desalted prior to mass spectrometry by rapid elution off a C3 column with a gradient of 5-95% isopropanol in water with 0.1% formic acid.

Crystallization: Protein was buffered in 50 mM HEPES pH 7.5, 300 mM NaCl, 5% glycerol and 5mM DTT. The protein was concentrated to 2.75 mg/ml (calculated using an extinction coefficient of 14440). Crystals were grown at 20 °C in 150 nl sitting drops mixing 75 nl protein solution with 75 nl of a reservoir solution containing 1.6M Magnesium Sulfate, 0.1M MES pH 6.5. On mounting crystals were cryoprotected with mother liquor plus 25% ethylene glycol before transfer to liquid nitrogen.

Data Collection: Resolution: 2.70Å
X-ray source: Diamond Light Source, station I03