KCTD17 (potassium channel tetramerization domain containing 17)

PDB Code: 5A6R

Material and Methods

Entry Clone Source: MGC

Entry Clone Accession: BC025403

SGC Construct ID: KCTD17A-c001

Entry clone accession/ sequence:

ATGAGGATGGAGGCCGGGGAGGCAGCGCCGCCGGCGGGGGCGGGCGGCCGCGCCGCAGGCGGCT
GGGGCAAGTGGGTG
CGGCTCAACGTGGGGGGCACGGTGTTCCTGACCACCCGGCAGACGCTGTGCCGCGAGCAGAAGT
CCTTCCTCAGCCGC
CTGTGCCAGGGGGAAGAGCTGCAGTCGGACCGGGATGAGACCGGGGCCTACCTCATTGACCGTG
ACCCCACCTACTTC
GGGCCCATCCTGAACTTCCTCCGGCATGGCAAGCTGGTGCTGGACAAGGACATGGCTGAGGAGG
GGGTCCTGGAGGAA
GCCGAGTTCTACAACATCGGCCCGCTGATCCGCATCATCAAAGACCGGATGGAAGAGAAGGACT
ACACGGTCACCCAG
GTCCCACCCAAGCATGTGTACCGCGTGCTGCAGTGCCAGGAGGAGGAGCTCACGCAAATGGTCT
CCACCATGTCTGAT
GGCTGGCGCTTCGAGCAGCTGGTGAACATCGGCTCCTCCTACAACTACGGCAGCGAGGACCAGG
CAGAGTTCCTGTGT
GTGGTGTCCAAGGAGCTCCACAGCACCCCAAACGGGCTGAGCTCAGAGTCCAGCCGCAAAACCA
AGAGCACGGAGGAG
CAGCTGGAGGAGCAGCAGCAGCAGGAGGAGGAGGTGGAGGAGGTGGAGGTGGAACAGGTGCAGG
TGGAGGCAGATGCA
CAGGAGAAAGGTTCCCGTCCGCACCCTCTCAGACCTGAGGCTGAGCTTGCAGTGAGGGCTTCTC
CTCGGCCCCTCGCC
CGCCCCCAGAGCTGCCATCCCTGCTGTTACAAGCCAGAGGCACCCGGATGTGAGGCCCCAGATC
ACCTCCAGGGACTT
GGGGTTCCCATCTGA

Expressed protein sequence:
MHHHHHHSSGVDLGTENLYFQSMGAG
GRAAGGWGKWVRLNVGGTVFLTTRQT
LCREQKSFLSRLCQGEELQSDRDETG
AYLIDRDPTYFGPILNFLRHGKLVLD
KDMAEEGVLEEAEFYNIGPLIRIIKD
RMEEK

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 was grown overnight at 37°C shaking at 200 RPM. 1L LB media with 50 µg/ml kanamycin was inoculated with the starter culture. The 1L culture was incubated at 37°C, 170 RPM shaking,

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 15 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 for a total of 15 minutes. The DNA was precipitated using 0.15% PEI (polyethyleneimine) pH 8. The cell lysate was spun down by centrifugation at 21.0K 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: 0.1mg 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, 5% glycerol

Procedure: Prior to applying the protein, the S75 16/60 column was washed and equilibrated with gel filtration buffer. The protein was concentrated to <5 ml using an Amicon Ultra-15 10 kD cutoff spin concentrator. The concentrated protein was directly applied onto the equilibrated S75 16/60 column, and run at a flow-rate of 0.8 ml/min. Fractions containing the protein were pooled together and concentrated using a 10kD cutoff Amicon spin concentrator.

Column 3: Ni-Affinity Chromatography (Rebind). 1 ml of 50 % Ni-sepharose slurry was applied onto a 1.5 x 10 cm column. The column was equilibrated with binding buffer (10ml).

Buffer:

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

Procedure: Pooled protein from gel filtration was flowed over bed of Ni-NTA resin. Flow through was collected. 2 x 1ml wash fractions were additionally collected and 2mM DTT was added to each fraction.

Column 4: Ion exchange chromatography - 5ml HiTrap Q.

Buffer:

Low salt buffer: 50 mM HEPES pH 7.5

High salt buffer: 50 mM HEPES pH 7.5, 1M NaCl

Procedure: Sample was concentrated to 5 ml and then diluted into low salt buffer before applying to a 5 ml HiTrap Q column equilibrated with low salt buffer. Sample was eluted using

a gradient of 0-1M NaCl at an NaCl concentration of around 900 mM.

Mass spec characterization: The purified protein had an experimental mass of N (after TEV cleavage), that is within the error of the expected mass of 12881.7 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 from Ion Exchange Chromatography was concentrated with addition of 5 mM Arginine and 5mM Glutamate to 30 mg/ml. Crystals were grown at 4 °C in 150 nl sitting drops mixing 75 nl protein solution with 75 nl of a reservoir solution containing 18% MPD, 0.1M Tris pH 8.5. On mounting, crystals were cryoprotected with mother liquor plus 20% ethylene glycol before transfer to liquid nitrogen.

Data Collection: Resolution: 2.85Å
X-ray source: Diamond Light Source, station I03