KCTD16 (potassium channel tetramerization domain containing 16)

PDB Code: 5A15

Material and Methods

Entry Clone Source: MGC

Entry Clone Accession: BC113435

SGC Construct ID: KCTD16A-c004

Entry clone accession/ sequence:

ATGGCTCTGAGTGGAAACTGTAGTCGTTATTATCCTCGAGAACAAGGGTCCGCAGTTCCCAACT
CCTTCCCTGAGGTG
GTAGAGCTGAATGTCGGGGGTCAAGTTTATTTTACTCGCCATTCCACATTGATAAGCATCCCTC
ATTCCCTCCTGTGG
AAAATGTTTTCCCCAAAGAGAGACACGGCTAATGATCTAGCCAAGGACTCCAAGGGAAGGTTTT
TCATTGACAGAGAT
GGATTCTTGTTCCGTTATATTCTGGACTATCTCAGGGACAGGCAGGTGGTCCTGCCTGATCACT
TTCCAGAAAAAGGA
AGACTGAAAAGGGAAGCTGAATACTTCCAGCTCCCAGACTTGGTCAAACTCCTGACCCCCGATG
AAATCAAGCAAAGC
CCAGATGAATTCTGCCACAGTGACTTTGAAGATGCCTCCCAAGGAAGCGACACAAGAATCTGCC
CCCCTTCCTCCCTG
CTCCCTGCCGACCGCAAGTGGGGTTTCATTACTGTGGGTTACAGAGGATCCTGCACCTTGGGCA
GAGAGGGACAGGCA
GATGCCAAGTTTCGGAGAGTTCCCCGGATTTTGGTTTGTGGAAGGATTTCCTTGGCAAAAGAAG
TCTTTGGAGAAACT
TTGAATGAAAGCAGAGACCCTGATCGAGCCCCAGAAAGATACACCTCCAGATTTTATCTCAAAT
TCAAGCACCTGGAA
AGGGCTTTTGATATGTTGTCAGAGTGTGGATTCCACATGGTGGCCTGTAACTCATCGGTGACAG
CATCTTTCATCAAC
CAATATACAGATGACAAGATCTGGTCAAGCTACACTGAATATGTCTTCTACCGTGAGCCTTCCA
GATGGTCACCCTCA
CACTGCGATTGCTGCTGCAAGAATGGCAAAGGTGACAAAGAAGGGGAGAGCGGCACGTCTTGCA
ATGACCTCTCCACA
TCTAGCTGCGACAGCCAGTCTGAGGCCAGCTCTCCCCAGGAGACGGTCATCTGTGGTCCCGTGA
CACGCCAGACCAAC
ATCCAGACTCTGGACCGTCCCATCAAGAAGGGCCCTGTCCAGCTGATCCAACAGTCAGAGATGC
GGCGGAAAAGCGAC
TTACTCCGGACTCTGACTTCAGGCTCCAGGGAATCGAACATGAGCAGCAAAAAAAAAGCTGTTA
AAGAAAAGCTCTCA
ATTGAGGAGGAGCTGGAGAAATGTATCCAGGATTTCCTAAAAATCAAAATTCCAGATCGGTTTC
CTGAGAGAAAACAT
CCTTGGCAATCTGAACTTTTAAGGAAGTATCATCTATAA

Expressed protein sequence:

MHHHHHHSSGVDLGTENLYFQSMGSA
VPNSFPEVVELNVGGQVYFTRHSTLI
SIPHSLLWKMFSPKRDTANDLAKDSK
GRFFIDRDGFLFRYILDYLRDRQVVL
PDHFPEKGRLKREAEYFQLPDLVKLL
TPDEIKQSPDE

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 7 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: 0.1mg of TEV protease was added to the Ni-eluted protein to remove the tag.

Column 2: Size Exclusion Chromatography - S200 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 S200 16/60 column was washed and equilibrated with gel filtration buffer. The protein was concentrated to 5 ml using an Amicon Ultra-15 filter. The concentrated protein was directly applied onto the equilibrated S200 16/60 column, and run at a flow-rate of 1 ml/min. Fractions containing the protein were pooled together and concentrated using a 3kD cutoff Amicon spin concentrator.

Mass spec characterization: The purified protein had an experimental mass of 13943.3 (after TEV cleavage), that is within the error of the expected mass of 13942.9 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 0.5mM TCEP. The protein was concentrated to 13 mg/ml (calculated using an extinction coefficient of 11460). Crystals were grown at 20 °C in 150 nl sitting drops mixing 75 nl protein solution with 75 nl of a reservoir solution containing 21% PEG3350, 0.1M bis-tris pH 6.0. On mounting, crystals were cryoprotected with mother liquor plus 25% ethylene glycol before transfer to liquid nitrogen.
Data Collection: Resolution: 2.76Å **X-ray source:**Diamond Light Source, station I03