Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5199628

SGC Construct ID: MAPK11A-c007

GenBank GI number: gi|20128774

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Amplified construct sequence:

```
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGCGCGCCGGCTTCTAC
CGGCAGGAGCTGAACAAGACCGTGTGGGA
GGTGCCGCAGCGGCTGCAGGGGCTGCGCC
CGGTGGGCTCCGGCGCCTACGGCTCCGTC
TGTTCGGCCTACGACGCCCGGCTGCGCCA
GAAGGTGGCGGTGAAGAAGCTGTCGCGCC
CCTTCCAGTCGCTGATCCACGCGCGCAGA
ACGTACCGGGAGCTGCGGCTGCTCAAGCA
CCTGAAGCACGAGAACGTCATCGGGCTTC
TGGACGTCTTCACGCCGGCCACGTCCATC
GAGGACTTCAGCGAAGTGTACTTGGTGAC
CACCCTGATGGGCGCCGACCTGAACAACA
TCGTCAAGTGCCAGGCGCTGAGCGACGAG
CACGTTCAATTCCTGGTTTACCAGCTGCT
GCGCGGGCTGAAGTACATCCACTCGGCCG
GGATCATCCACCGGGACCTGAAGCCCAGC
AACGTGGCTGTGAACGAGGACTGTGAGCT
CAGGATCCTGGATTTCGGGCTGGCGCGCC
AGGCGGACGAGGAGATGACCGGCTATGTG
GCCACGCGCTGGTACCGGGCACCTGAGAT
CATGCTCAACTGGATGCATTACAACCAAA
CAGTGGATATCTGGTCCGTGGGCTGCATC
ATGGCTGAGCTGCTCCAGGGCAAGGCCCT
CTTCCCGGGAAGCGACTACATTGACCAGC
TGAAGCGCATCATGGAAGTGGTGGGCACA
CCCAGCCCTGAGGTTCTGGCAAAAATCTC
CTCAGAACACGCCCGGACATATATCCAGT
CCCTGCCCCCCATGCCCCAGAAGGACCTG
AGCAGCATCTTCCGTGGAGCCAACCCCCT
GGCCATAGACCTCCTTGGAAGGATGCTGG
TGCTGGACAGTGACCAGAGGGTCAGTGCA
GCTGAGGCACTGGCCCACGCCTACTTCAG
CCAGTACCACGACCCCGAGGATGAGCCAG
AGGCCGAGCCATATGATGAGAGCGTTGAG
GCCAAGGAGCGCACGCTGGAGGAGTGGAA
GGAGCTCACTTACCAGGAAGTCCTCAGCT
TCAAGCCCTGACAGTAAAGGTGGATACGG
ATCCGAA
```

Tags and additions: Cleavable N-terminal His6 tag.

Final protein sequence:

```
mhhhhhhssgvdlgtenlyfq^sMRAGFY
RQELNKTVWEVPQRLQGLRPVGSGAYGSV
CSAYDARLRQKVAVKKLSRPFQSLIHARR
TYRELRLLKHLKHENVIGLLDVFTPATSI
EDFSEVYLVTTLMGADLNNIVKCQALSDE
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HVQFLVYQLLRGLKYIHSAGIIHRDLKPS
NVAVNEDCELRILDFGLARQADEEMTGYV
ATRWYRAPEIMLNWMHYNQTVDIWSVGCI
MAELLQGKALFPGSDYIDQLKRIMEVVGT
PSPEVLAKISSEHARTYIQSLPPMPQKDL
SSIFRGANPLAIDLLGRMLVLDSDQRVSA
AEALAHAYFSQYHDPEDEPEAEPYDESVE
AKERTLEEWKELTYQEVLSFKP

^ TEV cleave site

Host: BL21 (DE3)R3 (Phage resistant strain)

Growth medium, induction protocol: 1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 litre of LB containing 50 µg/ml kanamycin. Cultures were grown at 37°C until the OD_{600} reached ~0.3 then the temperature was adjusted to 18°C. Expression was induced for overnight using 1 mM IPTG at an OD_{600} of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. **Binding buffer**: 50 mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol; 0.5 mM TCEP.

Extraction buffer, extraction method: Frozen pellets were thawed and cells lysed using a high pressure cell disrupter. PEI (polyethyleneimine) was added to the lysate to a final concentration of 0.15 % and the lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification.

Column 1: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Buffers : **Binding buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5 mM TCEP; **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol, 0.5 mM TCEP; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole , 5% Glycerol, 0.5 mM TCEP.

Procedure: The cleared lysate was loaded by gravity flow on the Ni-NTA column. The column was then washed with 100 ml binding buffer and 100 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250 mM). Fractions were collected until essentially all protein was eluted, and 10 mM DTT was added.

Enzymatic treatment: The N-terminal His tag was cleaved by overnight incubation at 4°C with TEV protease. Cleaved products and TEV protease were removed by binding to Ni-NTA after buffer exchange to 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP using a 10 kDa cut-off concentrator.

Column 2: Size Exclusion Chromatography. Superdex S200 16/60 HiLoad.

Buffers: 10 mM HEPES, pH 7.5; 100 mM NaCl, 10 mM DTT

Procedure: The protein was concentrated and applied to an S200 16/60 HiLoad gel filtration column equilibrated in 10 mM HEPES, pH 7.5; 100 mM NaCl, 10 mM DTT using either an ÄKTA prime or ÄKTA express system.

Mass spectrometry characterization: LC- ESI -MS TOF gave a measured mass of 39704 for this construct as predicted from the sequence of this protein.

Protein concentration: Protein was concentrated to 11.8 mg/ml using an Amicon 10 kDa cut-off concentrator.

Crystallization: Diffraction quality crystals were grown at 4°C in 150 nl drops from a 1:1 ratio of protein and reservoir solution (20% PEG 3350; 0.1M citrate pH 5.5).

Data Collection: Crystals were cryo-protected using 25% EtGly; **Resolution:** 1.9 Å resolution limit; **X-ray source:** Diffraction data were collected at the Diamond beamline I04.