Entry Clone Source: Origene

Entry Clone Accession: NM_016542

SGC Construct ID: MST4A-c202

GenBank GI number: gi|15011880

Vector: "pLIC-SGC1". Details [PDF]; Sequence [FASTA] or [GenBank]

Amplified DNA sequence:

```
TACTTCCAATCCATGGCCCACTCGCCGGT
GGCTGTCCAAGTGCCTGGGATGCAGAATA
ACATAGCTGATCCAGAAGAACTGTTCACA
AAATTAGAGCGCATTGGGAAAGGCTCATT
TGGGGAAGTTTTCAAAGGAATTGATAACC
GTACCCAGCAAGTCGTTGCTATTAAAATC
ATAGACCTTGAGGAAGCCGAAGATGAAAT
AGAAGACATTCAGCAAGAAATAACTGTCT
TGAGTCAATGTGACAGCTCATATGTAACA
AAATACTATGGGTCATATTTAAAGGGGTC
TAAATTATGGATAATAATGGAATACCTGG
GCGGTGGTTCAGCACTGGATCTTCTTCGA
GCTGGTCCATTTGATGAGTTCCAGATTGC
TACCATGCTAAAGGAAATTTTAAAAGGTC
TGGACTATCTGCATTCAGAAAAGAAAATT
CACCGAGACATAAAAGCTGCCAATGTCTT
GCTCTCAGAACAAGGAGATGTTAAACTTG
CTGATTTTGGAGTTGCTGGTCAGCTGACA
GATACACAGATTAAAAGAAATACCTTTGT
GGGAACTCCATTTTGGATGGCTCCTGAAG
TTATTCAACAGTCAGCTTATGACTCAAAA
GCTGACATTTGGTCATTGGGAATTACTGC
TATTGAACTAGCCAAGGGAGAGCCACCTA
ACTCCGATATGCATCCAATGAGAGTTCTG
TTTCTTATTCCCAAAAACAATCCTCCAAC
TCTTGTTGGAGACTTTACTAAGTCTTTTA
AGGAGTTTATTGATGCTTGCCTGAACAAA
GATCCATCATTTCGTCCTACAGCAAAAGA
ACTTCTGAAACACAAATTCATTGTAAAAA
ATTCAAAGAAGACTTCTTATCTGACTGAA
CTGATAGATCGTTTTAAGAGATGGAAGGC
AGAAGGACACAGTTGACAGTAAAGGTGGA
TA
```

Tags and additions: Tag sequence: *Cleavable N-terminal His 6 tag.

Final protein sequence (tag sequence in lowercase):

```
mhhhhhhssgvdlgtenlyfq^sMAHSPV
AVQVPGMQNNIADPEELFTKLERIGKGSF
GEVFKGIDNRTQQVVAIKIIDLEEAEDEI
EDIQQEITVLSQCDSSYVTKYYGSYLKGS
KLWIIMEYLGGGSALDLLRAGPFDEFQIA
TMLKEILKGLDYLHSEKKIHRDIKAANVL
LSEQGDVKLADFGVAGQLTDTQIKRNTFV
GTPFWMAPEVIQQSAYDSKADIWSLGITA
IELAKGEPPNSDMHPMRVLFLIPKNNPPT
LVGDFTKSFKEFIDACLNKDPSFRPTAKE
LLKHKFIVKNSKKTSYLTELIDRFKRWKA
EGHS
```

^ TEV cleavage site

Host: BL21(DE3)-R3-pRARE2

Expression protocol: Transformed 50 µl competent BL-21 (DE3) phage resistant cells with 10 µl of the plasmid DNA and plated out onto LB plate plus 100 µg/ml ampicilin. The next day colonies were picked out into fresh deep well blocks containing 1 ml TB + 50 µg/ml ampicilin which were grown overnight and glycerol stocks were prepared by adding 333 ml of 60 % glycerol to 1 ml of cell suspension, which were stored at -80°C to be used for future scale up preparations.
Cells were grown at 37°C until the OD_{600} reached ~0.5. After that the temperature was lowered to 18°C. Protein production was induced with 1mM IPTG and recombinant MST4 was expressed at that temperature over night. The next day cells were harvested by centrifugation at 4000 rpm for 20 minutes. The cell pellet was stored at -20°C degrees.

Lysis and Ni-affinity chromatography: Buffers: Binding buffer: 50 mM HEPES pH 7.5, 300mM NaCl, 20 mM Imidazole; **Wash buffer 1:** 50 mM HEPES pH 7.5, 1M NaCl, 20mM Imidazole; **Wash buffer 2**:as for lysis buffer; **Elution buffer:** 50mM HEPES pH 7.5, 300mM NaCl, 200 mM Imidazole.

Procedure: The cell pellet (about 5g) was re-suspended in one volume (about 30 ml) of binding buffer. The re-suspended cells were lysed by sonication. The lysate was cleared of DNA and cell debris by centrifugation at 20,000 rpm (4°C).
5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 100 ml binding buffer. The lysate was applied to the column and was subsequently washed with 50 ml wash buffer 1 and 2. MST4 was eluted with 25 mls of elution buffer. The eluted protein was collected and analyzed by SDS-PAGE. DTT was added to the protein sample to a final concentration of 5 mM. The N-terminal his6-tag was removed by the addition of approximately 100 mg of TEV protease and incubated at 4°C overnight. Kinase phosphorylation was removed by l-phosphatase in the presence of 50 mM $MnCl_2$.

Column 2: Size exclusion chromatography HiLoad 16/60 Superdex 200

SEC-Buffers: 50 mM Hepes, pH 7.5, 300 mM NaCl, 5 mM DTT.

Procedure: The Tev cleaved eluted protein was concentrated by ultrafiltration (using a 10kDa cutoff ultrafiltration unit) The sample was then loaded and fractionated at 0.8 ml/min, on a HiLoad 16/60 Superdex 200 column prequilibrated with SEC Buffer. Eluted fractions were 95% pure as judged by SDS-PAGE. The eluted fractions were concentrated to 11.2 mg/ml using ultarfiltration (as above).

Mass spec characterization: ESI-MS revealed that the protein had the expected mass 33818 Da.

Protein concentration: 11.2 mg/ml in SEC buffer using a centricon with a 10kDa cut off.

Crystallization: MST4 was crystallized at 4°C using the sitting-drop vapor diffusion method. Diffraction quality crystals were obtained by mixing 200 nl of protein solution (5.9 mg/ml) with 100 nl of reservoir solution comprising 10-16% w/v PEG3350; 0.005M $CdCl_2$ and 0.1M HEPES pH 7.0. Crystal growth was encouraged by the addition of 20nl of a concentrated seed stock to the unequilibrated drops.

Data Collection: Crystals were flash frozen in liquid nitrogen using the buffer used for crystallization supplemented with 20% PEG400. Diffraction data were collected to 2.35 Å on beamline X10SA at the Swiss light source.