Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5205478

SGC Construct ID: CAMK4A-c007

GenBank GI number: gi|4502557

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

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

Final protein sequence (tag sequence in lowercase):

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mhhhhhhssgvdlgtenlyfq*sMSSVTA
SAAPGTASLVPDYWIDGSNRDALSDFFEV
ESELGRGATSIVYRCKQKGTQKPYALKVL
KKTVDKKIVRTEIGVLLRLSHPNIIKLKE
IFETPTEISLVLELVTGGELFDRIVEKGY
YSERDAADAVKQILEAVAYLHENGIVHRD
LKPENLLYATPAPDAPLKIADFGLSKIVE
HQVLMKTVCGTPGYCAPEILRGCAYGPEV
DMWSVGIITYILLCGFEPFYDERGDQFMF
RRILNCEYYFISPWWDEVSLNAKDLVRKL
IVLDPKKRLTTFQALQHPWVTGKAANFVH
MDTAQKKLQEFNARRKLKAAVKAVVASSR
LG
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Amplified DNA sequence:

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TACTTCCAATCCATGTCTTCGGTCACCGC
CAGTGCGGCCCCGGGGACCGCGAGCCTCG
TCCCGGATTACTGGATCGACGGCTCCAAC
AGGGATGCGCTGAGCGATTTCTTCGAGGT
GGAGTCGGAGCTGGGACGGGGTGCTACAT
CCATTGTGTACAGATGCAAACAGAAGGGG
ACCCAGAAGCCTTATGCTCTCAAAGTGTT
AAAGAAAACAGTGGACAAAAAAATCGTAA
GAACTGAGATAGGAGTTCTTCTTCGCCTC
TCACATCCAAACATTATAAAACTTAAAGA
GATATTTGAAACCCCTACAGAAATCAGTC
TGGTCCTAGAACTCGTCACAGGAGGAGAA
CTGTTTGATAGGATTGTGGAAAAGGGATA
TTACAGTGAGCGAGATGCTGCAGATGCCG
TTAAACAAATCCTGGAGGCAGTTGCTTAT
CTACATGAAAATGGGATTGTCCATCGTGA
TCTCAAACCAGAGAATCTTCTTTATGCAA
CTCCAGCCCCAGATGCACCACTCAAAATC
GCTGATTTTGGACTCTCTAAAATTGTGGA
ACATCAAGTGCTCATGAAGACAGTATGTG
GAACCCCAGGGTACTGCGCACCTGAAATT
CTTAGAGGTTGTGCCTATGGACCTGAGGT
GGACATGTGGTCTGTAGGAATAATCACCT
ACATCTTACTTTGTGGATTTGAACCATTC
TATGATGAAAGAGGCGATCAGTTCATGTT
CAGGAGAATTCTGAATTGTGAATATTACT
TTATCTCCCCCTGGTGGGATGAAGTATCT
CTAAATGCCAAGGACTTGGTCAGAAAATT
AATTGTTTTGGATCCAAAGAAACGGCTGA
CTACATTTCAAGCTCTCCAGCATCCGTGG
GTCACAGGTAAAGCAGCCAATTTTGTACA
CATGGATACCGCTCAAAAGAAGCTCCAAG
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AATTCAATGCCCGGCGTAAGCTTAAGGCA
GCGGTGAAGGCTGTGGTGGCCTCTTCCCG
CCTGGGATGACAGTAAAGGTGGATA

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 50 µg/ml kanamycin. The next day colonies were picked out into fresh deep well blocks containing 1 ml TB + 50 µg/ml kanamycin which were grown overnight and glycerol stocks were prepared by adding 333 µl of 60 % glycerol to 1 ml of cell suspension, which were stored at -80°C to be used for future scale up preparations.
The glycerol stock was used to inoculate 10 ml of LB supplemented with 50 µg/ml kanamycin and 35 µg/ml chloramphenicol. This starter culture was grown overnight at 37°C and used to inoculate a 2 liter culture in the same medium. The culture was 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 CAMK4A 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.

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. CAMK4A 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 CAMK4A 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 36806 Da.

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

Crystallization: CAMK4 was crystallized at 4°C using the sitting-drop vapor diffusion method. Diffraction quality crystals were obtained by mixing 150 nl of protein solution with 50 nl of 17% (w/v) PEG 10K; 0.10M (NH_4)(ac); 0.1M BIS-TRIS pH 5.5.

Data Collection: Crystals were flash frozen in liquid nitrogen. Diffraction data were collected to 2.4 Å at the Swiss light source beam-line X10SA at a single wavelength of 0.9999 Å.