Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5178265

SGC Construct ID: CAMK2DA-c021

GenBank GI number: gi|26667183

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

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

Final protein sequence: (lowercase refers to tag sequence)
mhhhhhhssgvdlgtenlyfqs*SMSNTTI
EDEDVKARKQEIIKVTEQLIEAINNGDFEA
YTKICDPGLTAFEPEALGNLVEGMDFHRFY
FENALSKSNKPIHTIILNPHVHLVGDDAAC
IAYIRLTQYMDGSGMPKTMQSEETRVWHRR
DGKWQNVHFHRSGSPTV

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 ml 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 CAMK2D 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 DNA was by a 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. CAMK2DA 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.

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 CAMK2DA protein was concentrated by ultrafiltration (using a 10kDa cutoff ultrafiltration unit) The sample was then loaded and fractionated at 1.0 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 15.25 mg/ml using ultarfiltration (as above). The protein was aliquoted into small samples and snap frozen in liquid nitrogen. The Protein was subsequently stored at -80°C. Some crystal trials were done with protein that had been repurified on a size exclusion column as previously indicated. No difference in quality of the protein was determined between sample protein that was repurified on an SEC 200 column following storage at -80°C and protein that was used directly following thawing without further purification.

Mass spec characterization: ESI-MS revealed that the protein had the expected mass 16392.83.
Protein concentration: 15.3 mg/ml in SEC buffer using a centricon with a 10kDa cut off.
Crystallization: CAMK2D was crystallized at 4°C using the sitting-drop vapor diffusion method. Diffraction quality crystals were obtained by mixing 75 nl of protein solution with 75 nl of 0.1M $CdCl_2$; 0.1M acetate pH 4.6; 30% PEG 400.
Data Collection: Crystals were flash frozen in liquid nitrogen. Diffraction data were collected to 2.6 Å at the Swiss light source beam-line X10SA at a single wavelength of 0.9999 Å.