Entry Clone Source: Origene

Entry Clone Accession: NM_015981 Variant

SGC Construct ID: CAMK2AA-c026

GenBank GI number: gi|25952114

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

Tags and additions: Tag sequence: *Cleavable N-terminal His6 tag.

Expressed sequence: (tag sequence in lowercase):
mhhhhhhssgvdlgtenlyfq*SMYQLFE
ELGKGAFSVVRRCVKVLAGQEYAAKIINT
KKLSARDHQKLEREARICRLLKHPNIVRL
HDSISEEGHHYLIFDLVTGGELFEDIVAR
EYYSEADASHCIQQILEAVLHCHQMGVVH
RDLKPENLLLASKLKGAAVKLADFGLAIE
VEGEQQAWFGFAGTPGYLSPEVLRKDPYG
KPVDLWACGVILYILLVGYPPFWDEDQHR
LYQQIKAGAYDFPSPEWDTVTPEAKDLIN
KMLTINPSKRITAAEALKHPWISHRSTVA
SCMHRQETVDCLKKFNARRKLKGA

Host: BL21 (DE3) Rosetta-*Phage resistant*

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 and 35 µg/ml chloramphenicol. The next day colonies were picked into fresh deep well blocks containing 1 ml LB + 50 µg/ml kanamycin and 35 µg/ml chloramphenicol. Cultures 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 and 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 1 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 CAMK2AA was expressed at that temperature over night. The next day cells were harvested by centrifugation at 4000 rpm for 15 minutes. The cell pellet was stored at -80°C degrees.

Lysis andNi-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, 150 mM Imidazole.

Procedure: The cell pellet (38 g) was re-suspended in one volume (38 ml) of binding buffer. The re-suspended cells were lysed by sonication. The lysate was cleared DNA was by a centrifugation at 17,000 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. CAMK2AA 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 10mM. The N-terminal his6-tag was not cleaved.

Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)

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

Procedure: The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 ml using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 0.8 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.
Mass spec characterization: ESI-MS revealed that the protein had the expected mass of 35585 Da for the uncleaved and unphosphorylated protein.
Protein concentration: 12 mg/ml in SEC buffer using a centricon with a 10kDa cut off
Crystallization: CAMK2AA was crystallized at 4°C using the sitting-drop vapor diffusion method at 10 mg/ml in the presence of 1 mM IDR E804**,** a cell-permeable indirubin derivative (CalBioChem #402081). Diffraction quality crystals were obtained by mixing 150 nl of protein solution with 50 nl of 12% (w/v) PEG6000 at pH 8.5 in Tris buffer.
Data Collection: Crystals were flash frozen in liquid nitrogen using the crystallization buffer and 30% propylene glycol. Diffraction data were collected to 2.3 Å at the Swiss light source beam-line X10SA.