CAMK1D - Human Ca2+/calmodulin-dependent protein kinase I-delta

PDB:2JC6

Revision

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Entry Clone Accession:gi|9966875
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SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host: Rosetta (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq^sMARENG ESSSSWKKQAEDIKKIFEFKETLGTGAFS EVVLAEEKATGKLFAVKCIPKKALKGK
ES SIENEIAVLRKIKHENIVALEDIYESPNH LYLVMQLVSGGELFDRIVEKGFYTEKDAS TLIRQVLDAVYYLHRMGIVHRDLK
PENLL YYSQDEESKIMISDFGLSKMEGKGDVMST ACGTPGYVAPEVLAQKPYSKAVDCWSIGV IAYILLCGYPPFYDENDSKLF
EQILKAEY EFDSPYWDDISDSAKDFIRNLMEKDPNKR YTCEQAARHPWIAGDTALNKNIHESVSAQ IRKNFAKSKWRQAFNATA
VVRHMRKLHLG SSLDSSNA ^:cleavage site for TEV protease

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

Growth

Medium:
Antibiotics:
Procedure:3ml from a 50 ml overnight culture was used to inoculate each of two flasks containing 1 litre of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol. Cultures were grown at 37°C to an OD600 of ~0.4 and then cooled to 18°C. Expression was induced for 4 hours using 0.5mM 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: 50mM HEPES pH 7.5; 500 mM NaCl; 5% glycerol; 5 mM imidazole.

Purification

Procedure

Extraction

Procedure
Cell pellets in binding buffer plus 1 mM PMSF, 0.5 mM TCEP were lysed using sonication. The lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification. Prior to purification the lysate was passed through a DE52 column (10g/L resin) to remove DNA.
Concentration:
Ligand
MassSpec:
Crystallization: The inhibitor (5-Methyl-1H-pyrazol-3-yl)-(2-phenylquinazolin-4-yl)amine was added to the protein in 3-fold excess and the complex concentrated to 10 mg/ml. Crystals were obtained in sitting drops using the vapor diffusion method by mixing 75nl of the concentrated protein with 75nl of a well solution containing 0.1M citrate pH 5.6; 20% isopropanol; 20% PEG 4K. Crystals appeared after several days at 4°C.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution and 15% ethylene glycol and flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line X10 at a single wavelength (0.99 nm).
Data Processing: