

CAMK1GA: Human Ca²⁺/calmodulin-dependent protein kinase I-gamma

PDB:2JAM

Revision

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SGC Clone Accession:

Tag:Tag sequence: mhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.

Host:BL21 (DE3)

Construct

Prelude:

Sequence:

mhhhhhssgvdlgtenlyfq^sMQTTNI RKTFFMEVLGSGAFSEVFLVKQRLTGKL FALKCIKKSPAFRDSSLENEIAVLKKI
KH ENIVTLEDIYESTTHYYLVMQLVSGGELF DRILRGVYTEKDASLVIQQVLSAVKYLH ENGIVHRDLKPENLLYLTPEENSK
IMITD FGLSKMEQNGIMSTACGTPGYVAPEVLAQ KPYSKAVDCWSIGVITYILLCGYPPFYEE TESKLFEKIKEGYEFESPFW
DDISESAK DFICHLLEKDPNERYTCEKALSHPWIDGN TALHRDIYPSVSLQIQKNFAKSKWRQAFN AAADVHH

Vector:pNIC28-Bsa4

Growth

Medium:

Antibiotics:

Procedure:1ml from a 10 ml overnight culture containing 50 µg/ml kanamycine was used to inoculate 1 liter of LB media containing 100 µg/ml ampiciline. Cultures were grown at 37°C until the OD 600 reached ~0.3. After that the temperature was adjusted to 18°C. Expression was induced for 4 hours using 1mM IPTG at an OD600 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; 300 mM NaCl; 20 mM imidazole.

Purification

Procedure

Extraction

Procedure

Cell pellets were lysed using a high pressure cell disrupter. The lysate was centrifuged at 19,000 rpm for 60 minutes and the supernatant collected for purification.

Concentration:**Ligand****MassSpec:**

Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 10 mg/ml containing 1 mM 5-[(Z)- (5-Chloro- 2-oxo-1,2- dihydro-3H- indol-3-ylidene) methyl]- N-[2- (diethylamino) ethyl]-2,4- dimethyl-1H- pyrrole-3- carboxamide by mixing 100nl of the concentrated protein with 100nl of a well solution containing 0.20M Na(malonate); 0.1M BTPProp pH 7.5; 20.0% PEG 3350; 10.0% EtGly. Crystals appeared after several days at 4°C.

NMR Spectroscopy:

Data Collection: Crystals were cryo-protected using the well solution and 25% ethylene glycole and flash frozen in liquid nitrogen. Resolution: 1.8 Å. X-ray source: Diffraction data were collected at the SLS beam line X10 at a single wavelength (0.99 nm).

Data Processing: