

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

PDB:2JAM

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**gi|14196445

**Entry Clone Source:**MGC

**SGC Clone Accession:**

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

**Host:**BL21 (DE3)

## Construct

**Prelude:**

**Sequence:**

mhhhhhssgvdlgtenlyfq^sMQTTNI RKTIFMEVLGSGAFSEVFLVKQRLTGKL FALKCIKKSPAFRDSSLENEIAVLKKI  
KH ENIVTLEDIYESTTHYYLVMQLVSGGELF DRILERGVYTEKDASLVIQQVLSAVKYLH ENGIVHRDLKPENLLYLTPEENSK  
IMITD FGLSKMEQNGIMSTACGTPGYVAPEVLAQ KPYSKAVDCWSIGVITYILLCGYPPFYEE TESKLFEKIKEGYYEFESPFW  
DDISESAK DFICHLLEKDPNERYTCEKALSHPWIDGN TALHRDIYPSVSLQIQKNFAKSKWRQAFN AAAVVHH

**Vector:**pNIC28-Bsa4

## Growth

**Medium:**

**Antibiotics:**

**Procedure:** 1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 liter of LB media containing 100 µg/ml ampicilin. 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 BTProp 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:**