CSNK1G1A: Human casein kinase gamma 1

PDB:2CMW

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:
Tag:
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmRVGKKIGCGNFGELRLGKNLYTNEYVAIKLEPIKSRAPQLHLEYRFYKQLGSAGEGLPQVYYFG
PCGKYNAMVLELLGPSLEDLFDLCDRTFTLKTVLMIAIQLLSRMEYVHSKNLIYRDVKPENFLIGRQGNKKEHVIHIIDFGLAKEYI
DPETKKHIPYREHKSLTGTARYMSINTHLGKEQSRRDDLEALGHMFMYFLRGSLPWQGLKADTLKERYQKIGDTKRNTPIEALCENF
PEEMATYLRYVRRLDFFEKPDYEYLRTLFTDLFEKKGYTFDYAYDWVGRPIPTPVGSVHVDSGASAITRE

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:1 ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 liter of LB media containing 50 µg/ml kanamycin. Cultures were grown at 37°C until the OD 600 reached ~2.0. After that the temperature was adjusted to 20°C. Expression was induced for 4 hours using 1mM IPTG. The cells were collected by centrifugation and the pellet was frozen for until processed.

Purification

Procedure
Column 1: Ni-affinity chromatography.
Procedure: 5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 50 ml binding buffer. The lysate was applied to the column which was subsequently washed with 50 ml wash buffer 1 and 2. CSNK1G1 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 5mM. The N-terminal his 6 -tag was cleaved by incubating the protein overnight with TEV protease.

Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)
Procedure: The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 mls 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. CSNK1G1 eluted at 65 minutes corresponding to a retention time of a monomeric protein of that size. Eluted fractions were 95% pure as judged by SDS-PAGE.

Protein concentration: Centricon with a 10kDa cut off in SEC-buffer

Extraction

Procedure
25 ml of 50 mM HEPES pH 7.5, 300 mM NaCl, 5 mM NaPO4, 20 mM Imidazole. The cells were lysed by sonication for 3 min (40% max, 20 sec on 1 m in off).
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 10 mg/ml containing 1 mM 2-(2-Hydroxyethylamino)-6-(3-chloroanilino)-9-isopropylpurine by mixing 100nl of the concentrated protein with 100nl of a well solution containing 12% PEG10K, 0.05M ammonium acetate, 0.1M bis tris pH 5.5. Crystals appeared after 3 days at 4°C.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution and 20% PEG400 and flash frozen in liquid nitrogen. Diffraction data were collected at the SLS beam line X10 at a single wavelength (0.9765 Å).
Data Processing: