CSNK1G2

PDB:2C47

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|21314778
Entry Clone Source:synthetic DNA
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfqsmGPNFRVGKKIGCGNFGELRLGKNLYTNEYVAIKLEPIKSRAPQLHLEYRFYKQLSATEGVPQVY
YFGPCGKYNAMVLELLGPSLEDLFDLCDRTFTLKTVLMIAIQLITRMEYVHTKSLIYRDVKPENFLVGRPGTKRQHAIHIIDFGLAK
EYIDPETKKHIPYREHKSLTGTARYMSINTHLGKEQSRRDDLEALGHMFMYFLRGSLPWQGLKADTLKERYQKIGDTKRATPIEVLC
ENFPEEMATYLRYVRRLDFFEKPDYDYLRKLFTDLFDRS GFVFDYEYDWAGKPLPTPIGTVHTDLPSQPQLRD
```

Vector:pLIC- SGC1

Growth

Medium:
Antibiotics:
Procedure:1mL from a 10 mL overnight culture containing 100 µg/mL ampiciline was used to inoculate 1 liter of LB media containing 100 µg/mL ampiciline. Cultures were grown at 37°C until the OD600 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
Column 1: Ni-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. 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. CSNK1G2 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 his6-tag was cleaved by incubating the protein overnight with TEV protease.

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

SEC-Buffers: 50 mM Hepes, pH 7.5, 300 mM NaCl, 5 mM DTT. 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. CSNK1G2 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
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-Iodotubercidin by mixing 100nl of the concentrated protein with 100nl of a well solution containing 19% PEG10K, 0.2M magnesium sulfate, 0.1M cacodylate pH 7.3. Crystals appeared after 3 days at 4°C.
NMR Spectroscopy:
Data Collection:
Data Processing: