CSNK1G3

PDB:2CHL
Entry Clone Accession:NM_001031812
Entry Clone Source: Origene
Host: Rosetta-R3 (DE3)

Construct

Sequence:

mhhhhhhssgvdlgtenlyfqsmGVLMVG PNFRVGKKIGCGNFGELRLGKNLYTNEYV AIKLEPMKSRAPQLHLEYRFYKQLGSG DG IPQVYYFGPCGKYNAMVLELLGPSLEDLF DLCDRTFSLKTVLMIAIQLISRMEYVHSK NLIYRDVKPENFLIGRPGNKTQQV IHIID FALAKEYIDPETKKHIPYREHKSLTGTAR YMSINTHLGKEQSRRDDLEALGHMFMYFL RGSLPWQGLKADTLKERYQKI GDTKRATP IEVLCENFPEMATYLRYVRRLDFFEKPDY DYLRKLFTDLFDRKGYMFDYEYDWIGKQL PTPVGAVQQDPALSSNRE AHQHRDKMQQS KNQ

Vector:pNIC28-Bsa4

Growth

Procedure:1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 liter of TB media containing 50µ/ml kanamycin. Cultures were grown at 37degC until the OD 600 reached ~2.0. After that the temperature was adjusted to 25\'b0C. Expression was induced for 4 hours using 1mM IPTG. The cells were collected by centrifugation and the pellet were frozen.

Purification

Buffers
Binding buffer: 50 mM HEPES pH 7.5, 300mM NaCl,, 20 mM Imidazole.

Wash buffer: 50 mM HEPES pH 7.5, 1M NaCl, 20mM Imidazole.

Elution buffer: 50mM HEPES pH 7.5, 300mM NaCl, 150 mM Imidazole.

SEC-Buffers: 50 mM Hepes, pH 7.5, 300 mM NaCl, 5 mM DTT.
Procedure
Column 1: Ni-affinity chromatography.
The column was equilibrated with binding buffer. The lysate was applied to the column which was subsequently washed with wash buffer 1. CSNK1G3 was eluted with 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, shrimp alkaline phosphatase and lambda phosphatase.

Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)
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. CSNK1G3 eluted at a retention time corresponding to the monomeric protein. Eluted fractions were 95% pure as judged by SDS-PAGE, and confirmed by mass spectrometry as the unphosphorylated protein.

Extraction

Procedure
Cell pellets were resuspended in 50 ml binding buffer lysed using sonication . The lysate was centrifuged at 19,000 rpm for 60 minutes and the supernatant collected for purification.
Concentration: Centricon with a 10kDa cut off in SEC-buffer
Crystallization: Crystals were obtained using the vapor diffusion method and a protein concentration of 10 mg/ml containing and 1 mM of various inhibitors used by mixing 100nl of the concentrated protein with 100nl of a well solution
Data Collection: Resolution: 1.95 \'c5; X-ray source: Rotating anode, Rigaku FR-E superbright