

Entry clone source: Origene
Entry clone accession : NM_001031812
Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]
Sequence, tags and additions: mhhhhhssgvdltgtenlyfq*sMGVLMVGPnFRVGKKIG CGNFGELRLGKNLYTNEYVAIKLEPMKSRAPQLHLEYRFY KQLGSGDGIPQVYFPGCGKYNAMVLELLGPSLEDLFDLC DRTFSLKTVLMIAIQLISRMEYVHSKNLIYRDVKPENFLI GRPGNKtQQVIHIIDFALAKEYIDPETKKHIPYREHKSLT GTARYMSINTHLGKEQSRDDLEALGHMFMYFLRGSLPWQ GLKADTLKERYQKIGDTRATPIEVLCEMFEMATYLRVY RRLDFFEKPDYDLRKLFTDLFDRKGYMFDYEYDWIGKQL PTPVGAVQQDPALSSNREAHQHRDKMQQSKNQ TEV-cleavable (*) N-terminal his6 tag.
Host : BL-21(DE3)R3 phage resistant
Growth medium, induction protocol: 1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 liter of TB 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 25°C. Expression was induced for 4 hours using 1mM IPTG. The cells were collected by centrifugation and the pellet were frozen.
Extraction buffer, extraction method: 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.
Column 1 : Ni-affinity, HisTrap, 1 ml (GE/Amersham)
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. Elution buffer: 50mM HEPES pH 7.5, 300mM NaCl, 150 mM Imidazole.
Procedure: 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)
Buffers : Gel Filtration Buffer: 50 mM Hepes, pH 7.5, 300 mM NaCl, 5 mM DTT
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. 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.
Protein concentration: Centricon with a 10kDa cut off in SEC-buffer
Crystallisation & Data Collection: Crystals were obtained using the vapor diffusion method. di-substituted Diaminothiazole complexes were crystallized using a protein concentration of 16 mg/ml containing 1 mM of inhibitor. Drops were setup in 96 well sitting drop plates by mixing either 100nl of the concentrated protein with 50nl of a well solution containing 10% PEG10K, 0.2 M MgCl₂, 0.1M Tris pH 7.0 or 75 nl of protein solution with 75 nl of well solution. Diffraction data were collected at 1.30 Å

and 1.95 Å resolution from a crystal that was cryo-protected with 20% propylene glycole using the SLS beamline X10SA or an FRE X-ray generator (Rigaku) and a HTC detector, respectively.