Entry Clone Source: Origene

Entry Clone Accession: NM_001031812 Variant

SGC Construct ID: CSNK1G3A-c002

GenBank GI number: gi|4758080

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Sequence, tags and additions:

```
mhhhhhhssgvdlgtenlyfq*sMGVLMVGPNFRVGKKIG
CGNFGELRLGKNLYTNEYVAIKLEPMKSRAPQLHLEYRFY
KQLGSGDGIPQVYYFGPCGKYNAMVLELLGPSLEDLFDLC
DRTFSLKTVLMIAIQLISRMEYVHSKNLIYRDVKPENFLI
GRPGNKTQQVIHIIDFALAKEYIDPETKKHIPYREHKSLT
GTARYMSINTHLGKEQSRRDDLEALGHMFMYFLRGSLPWQ
GLKADTLKERYQKIGDTKRATPIEVLCENFPEMATYLRYV
RRLDFFEKPDYDYLRKLFTDLFDRKGYMFDYEYDWIGKQL
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.

The N-(cis-2-Aminocyclohexyl)-N-(3-chlorophenyl)-9-ethyl-9H-purine-2,6-diamine complex was crystallized using a protein concentration of 17 mg/ml containing 1 mM of inhibitor. Drops were setup in 96 well sitting drop plates by mixing 75nl of the concentrated protein with 75nl of a well solution containing 0.05M CaCl2; 0.1M TRIS pH 8.0; 20.0% PEG 6K; 10.0% EtGly. Diffraction data were collected at 2.0 Å resolution from a crystal that was directly frozen from the crystallization solution using a FRE X-ray generator (Rigaku) and a HTC detector.