CCNT2: Human Cyclin T2

PDB:2IVX

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|4502629
Entry Clone Source: Origene
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s TEV-cleavable (*) N-terminal his6 tag.
Host: Rosetta

Construct

Prelude: Note the expressed protein has three changes (shown bold) compared with gi|4502629.
Sequence:

mhhhhhhssgvdlgtenlyfqsLASGRGA SSRWFFTREQLENTPSRRCGVEADKELSC RQQAANLIQEMGQRLNVSQLTINTAIV
YM HRFYMHHSFTKFNKNIISSTALFLAAKVE EQARKLEHVIKVAHACLHPLEPLLDTKCD AYLQQTRELVILETIMLQTLGFEI
TIEHP HTDVVKCTQLVRASKDLAQTSYFMATNSL HLTTFCLQYKPTVIACVCIHLACKWSNWE IPVSTDGKHWWEYVDPTVTLE
LLDELTHE FLQILEKTPNRLKKIRNWRANQAARKPKV DGQVA

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:1 ml from a 10 ml overnight culture was used to inoculate each of three flasks containing 0.8 L of 2xTY media. Cultures were grown at 37°C until the OD600 reached ~0.7 before the temperature was adjusted to 18°C. Expression was induced for 5 hours using 0.1mM IPTG. The cells were collected by centrifugation and the pellets were frozen. All cultures contained 50 µg/ml kanamycin and 34 µg/ml chloramphenicol.

Purification

Procedure
Column 1: Ni-affinity chromatography.
5 ml of Ni-sepharose slurry was packed in a drip column and equilibrated with binding buffer. The lysate was applied to the column, washed with 100 ml of wash buffer before step elution with increasing imidazole. 10 mM DTT was added to the eluted protein. Fractions were analyzed by SDS-PAGE. The N-terminal his 6 -tag was cleaved by incubating the protein overnight with TEV protease.

Column 2: Size exclusion chromatography (Superdex S75, HiLoad 26/60)
The fractions eluted from the Ni-affinity chromatography were concentrated to ~4 mls using Amicon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 2 ml/min. CCNT2A eluted at a retention time corresponding to the monomeric protein. 10 mM DTT was added to the elution which was >95% pure as judged by SDS-PAGE, and confirmed by mass spectrometry.

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

Extraction

Procedure
Cell pellets were resuspended in 70 ml binding buffer containing 1mM PMSF and 0.5 mM TCEP. Lysis was performed by high pressure homogeniser. The lysate was centrifuged at 16,000 rpm for 40 minutes and the supernatant collected for purification. Prior to purification the lysate was passed through a DE52 column (10g/L resin) to remove DNA.
Concentration:
Ligand
MassSpec:
Crystallization: Native: Crystals were obtained at 4°C using the vapor diffusion method with 150 nl sitting drops. 10 mg/ml protein was mixed at a ratio of 1:2 with the precipitant solution consisting of 0.2M sodium formate, 0.1M BisTrisPropane, 20% PEG 3350, 10% EtGly. Seleno-methionine: SeMet labeled crystals grew from 0.2M sodium fluoride, 20% PEG 3350, 10% EtGly at 4°C.
NMR Spectroscopy:
Data Collection: Diffraction data were collected at the Swiss Light Source X10 beamline to 1.8Å resolution from a crystal that had been cryo-protected with 20% EtGly.
Data Processing: The structure was solved using SeMet-SAD data collected at 0.9794 Å wavelength at the Swiss Light Source X10 beamline.