CDPK1

PDB:3IGO

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:cgd3_920:MAC024-B07:C29724
Tag:N-terminal tag: MHHHHHHSSGRENLYFQG
Host:BL21(DE3)V2RpACYC-LIC+LamP-phosphatase

Construct

Prelude:M1-G70 truncated
Sequence:

```
TFAERYNIVCMLGKGSFGEVLKCKDRITQQEYAVKVINKASAKNKDTSTILREVELLKKLDHPNIMKLFEILEDSSSFYIVGELYTG
GELFDEIIKRKRFSEHDAARIIKQVFSGITYMHKHNIVHRDLKPENILLESKEKDCDIKIIDFGLSTCFQQNTKMKDRIGTAYYIAP
EVLRGTYDEKCDVWSAGVILYILLSGTPPFYGKNEYDILKRVETGKYAFDLPQWRTISDDAKDLIRKMLTFHPSLRITATQCLEHPW
IQKYSSETPTISDLPSLESAMTNIRQFQAEKKLAQAALLYMASKLTTLDETKQLTEIFRKLDTNNDGMLDRDELVRGYHEFMRLKGV
DSNSLIQNEGSTIEDQIDSLMPLLDMDGSGSIEYSEFIASAIDRTILLSRERMERAFKMFDKDGSGKISTKELFKLFSQADSSIQME
ELESIIEQVDNNKDGEVDFNEFVEMLQNFVRNE
```

Vector:pET15-MHL

Growth

Medium:TB
Antibiotics:
Procedure: Express plasmid in E. coli BL21(DE3)V2RpACYC-LIC+LamP-phosphatase on LB(Lauria broth) plate in the presence of carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL). A single colony was inoculated into 50 mL of TB with carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL) in a 250 mL shaking flask and incubated at 37 °C for overnight. Then the culture was transfer into 1.8 L of TB with carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL) and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of ~5, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

Purification

Procedure
Affinity column:The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with

Binding Buffer); and subsequently onto a 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 - 1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 - 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. TCEP was then added to 1 - 5 mM.

Gel filtration:The sample was loaded onto a Sephadex S200 26/60 column equilibrated with Crystal Buffer. The fractions from the peak corresponding to monomer protein were collected.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at 80 degC were thawed overnight at 4 °C on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with protease inhibitors, 0.5% CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.25 rotor at 24,000 rpms for 20 minutes at 10 ºC.
Concentration:protein concentration:29mg/ml
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 ºC in 20%peg3350, 0.2M di-NH4tart with ligand 2mM AMPPNP,2mM CaCl2,4mM MgCl2 and 6.25mM TCEP using the Sitting drop method.
NMR Spectroscopy:
Data Collection:
Data Processing: