CDPK6

PDB:3IS5

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:38.m00014:MAC03B-B07:C205909
Tag:
Host:BL21(DE3)V2RpACYC-LIC+LamP-phosphatase

Construct

Prelude:T112-A378 with N-terminal his tag
Sequence:

MHHHHHHSSGRENLYFQGTIDDLFIFKRKLGSGAFGDVHLVEERSSGLERVIKTINKDRSQVPMEQIEAEIEVLKSLDHPNIIKIFE
VFEDYHNMYIVMETCEGGELLERIVSAQARGKALSEGYVAELMKQMMNALAYFHSQHVVHKDLKPENILFQDTSPHSPIKIIDFGLA
ELFKSDEHSTNAAGTALYMAPEVFKRDVTFKCDIWSAGVVMYFLLTGCLPFTGTSLEEVQQKATYKEPNYAVECRPLTPQAVDLLKQ
MLTKDPERRPSAAQVLHHEWFKQA

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 25 mL of TB with carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL) in a 50 mL falcon tube and incubated with shaking at 250 rpm overnight at 37 °C. Then the culture was transfer into 1L of TB with carbenicillin(100mg/ml)+chloramphenicol (34 mg/mL) and 0.3 mL of antifoam (Sigma) in a 1 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. 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 Gel Filtration 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 80oC 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 sonicated for effective time 10 minutes(about 120 watts, pulsed 10s on, 10s off) 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:15mg/ml
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 ºC in 13.5%peg8K, 0.1M tris9.5 and 0.2M MgCl2 with 2mMAMPPNP,2mMCaCl2,4mM MgCl2 and 6.6mMTCEP using the Sitting drop method.
NMR Spectroscopy:
Data Collection:
Data Processing: