CLK3

PDB:2EU9

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_003983
Entry Clone Source:MGC
SGC Clone Accession:
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfq*s(m) . TEV-cleavable (*) N-terminal hexaHis tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqSMQSSKRSSRSVEDDKEGHLVCRIGDWLQERYEIVGNLGEGTFGKVVECLDHARGKSQVALKIIRN
VGKYREAARLEINVLKKIKEKDKENKFLCVLMSDWFNFHGHMCIAFELLGKNTFEFLKENNFQPYPLPHVRHMAYQLCHALRFLHEN
QLTHTDLKPENILFVNSEFETLYNEHKSCEEKSVKNTSIRVADFGSATFDHEHHTTIVATRHYRPPEVILELGWAQPCDVWSIGCIL
FEYYRGFTLFQTHENREHLVMMEKILGPIPSHMIHRTRKQKYFYKGGLVWDENSSDGRYVKENCKPLKSYMLQDSLEHVQLFDLMRR
MLEFDPAQRITLAEALLHPFFAGLTPEERSFHT

Vector:pLIC- SGC 1

Growth

Medium:
Antibiotics:
Procedure:1 ml from a 10 ml overnight culture in LB, 100 µg/ml ampicillin was used to inoculate 1 litre of LB medium containing 100 µg/ml ampicillin. Cultures were grown at 37°C until they reached an OD600 of 0.3 and then cooled to 18°C. Expression was induced for 4 hours using 1 mM IPTG at an OD600 of 0.6. The cells were collected by centrifugation, transferred to 50 ml tubes, resuspended in 30 ml binding buffer, and frozen. Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 50 mM L-Arg and L-Glu.

Purification

Procedure
Column 1: Ion exchange - Nucleic acid removal. DEAE cellulose (DE52, Whatman), 10 g of resin in 2.5 x 20 cm column. The resin was hydrated in 2.5M NaCl, then washed with 20 ml binding buffer prior to loading the sample.

Buffers: Binding buffer: 50 mM HEPES, 500 mM NaCl, 5% Glycerol, 50 mM L-Arg and L-Glu.

Procedure: Supernatant was applied at gravity flow, followed by a wash with 50 ml binding buffer. The column flow-through was collected.

Column 2: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Buffers : Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 50 mM L-Arg and L-Glu. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 20 mM Imidazole, 5% glycerol, 50 mM L-Arg and L-Glu. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 50 to 250 mM Imidazole , 5% Glycerol, 50 mM L-Arg and L-Glu.

Procedure: The flowthrough from column 1 was loaded by gravity flow on the Ni-NTA column. The column was then washed with 3 x 10 ml wash buffer at gravity flow. The protein was eluted by gravity flow by applying 5-ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 and 250 mM); fractions were collected until essentially all protein was eluted. After elution DTT was added to a final concentration of 10 mM.

Enzymatic treatment : (Dephosphorylation and His tag cleavage) Samples containing CLK3 were pooled and 20 µg GST-lambda phosphatase and 20 µg TEV protease added for overnight incubation at 4°C: protein solution contained 10 mM DTT and 0.05 mM MnCl 2

Column 3: Size Exclusion Chromatography

Buffers: Fractions containing CLK3 collected from IMAC were concentrated and directly applied to a S75 16/60 HiLoad gel filtration column equilibrated in 50 mM Hepes pH 7.5, 500 mM NaCl, 50 mM L-glutamic acid, 50 mM L-arginine

Procedure : AKTA-prime

Column 4: Anion Exchange Chromatography

Buffers: Fractions containing CLK3 collected from SEC were diluted to a final concentration of 50 mM HEPES pH 7.5, 50 mM NaCl and applied to a MonoQ 5/50 GL equilibrated in 50 mM Hepes pH 7.5, 50 mM NaCl. The potein was eluted using an NaCl gradient

Procedure : AKTA-express

Extraction

Procedure
The frozen cells were thawed on ice and binding buffer (plus 1 mM PMSF) added to a final volume of 50 ml. Cells were lysed using a high pressure cell disruptor. The lysate was centrifuged at 18,500 RPM for 50 minutes and the supernatant collected for purification.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown at 4°C in 150nl sitting drops mixing 75 nl of CLK3 11mg/ml in 50mM Hepes pH 7.5, 200mM NaCl,10mM DTT, with 75 nl of a solution containing 27%PEG 3350, 30 mM Ammonium acetate, 100 mM Bis Tris pH 5.5
NMR Spectroscopy:
Data Collection:
Data Processing: