CLK1

PDB:1Z57

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_004062
Entry Clone Source:MGC
SGC Clone Accession:
Tag: Tag sequence: MHHHHHHSSGVDLGTENLYFQ*S(M) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqSMHLICQSGDVLSARYEIVDTLGEGAFGKVVECIDHKAGGRHVAVKIVKNVDRYCEAARSEIQVLE
HLNTTDPNSTFRCVQMLEWFEHHGHICIVFELLGLSTYDFIKENGFLPFRLDHIRKMAYQICKSVNFLHSNKLTHTDLKPENILFVQ
SDYTEAYNPKIKRDERTLINPDIKVVDFGSATYDDEHHSTLVSTRHYRAPEVILALGWSQPCDVWSIGCILIEYYLGFTVFPTHDSK
EHLAMMERILGPLPKHMIQKTRKRKYFHHDRLDWDEHSSAGRYVSRACKPLKEFMLSQDVEHERLFDLIQKMLEYDPAKRITLREAL
KHPFFDLLKKSI

Vector:pLIC-SGC

Growth

Medium:
Antibiotics:
Procedure: Grow starter cultures from freshly transformed colonies in 10 ml LB, 0.1 mg/mL amp. This started culture was diluted 1:1000 in fresh media and was grown at 37 o C to a OD 600 of 0.3 and than transferred to 18 o C. Expression was induced at an OD 600 of 0.8 using 1 mM IPTG. Cells were harvested after 3h by centrifugation, transferred to 50-ml tubes, and frozen in liquid nitrogen.

Purification

Procedure
Ni affinity: HisTrap (1 ml) gravity column. Loading buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 10 mM imidazole, 0.5 mM TCEP, 5% glycerol. Wash buffer: 50 mM Hepes, pH 7.5, 500 mM NaCl, 50 mM imidazole, 5% glycerol. Elution buffer: 50 mM Hepesl, pH 7.5, 500 mM NaCl, 250 mM imidazole, 0.5 mM TCEP, 5% glycerol. The column was then washed with 10 volumes of Loading buffer, 10 volumes of wash buffer.

SEC: Fractions containing CLK1 collected from IMAC were concentrated and directly applied to a S75 column equilibrated in 50 mM Hepes pH 7.5, 500 m NaCl, 50 mM glutamate, 50 mM Arginine. Procedure: AKTA-prime.

Concentration: Centricons 10 kDa cut off concentrated in the presence of 1 mM 10Z-Hymenialdisine

Extraction

Procedure
Extraction buffer: 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM PMSF. The cell pellets (20 gr wet wt) were re-suspended in 50 ml extraction buffer, and lysed by a high pressure cell disrupter. Supernatant was centrifuged for 30 minutes at 20 rpm in a JA 20 rotor
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown at 4 ºC in 200nl sitting drops mixing 100 nl of CLK1 (6 mg/mL in 50mM Hepes pH 7.5, 100mM NaCl ,10mM DTT, 50 mM glutamate, 50 mM arginine) with 100 nl of a solution containing 20% PEG 6K, 0.1 M Bicine pH 9.0
NMR Spectroscopy:
Data Collection:
Data Processing: