DMPK1

PDB:2VD5

Revision

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Entry Clone Accession:gi|976144
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SGC Clone Accession:IMAGE:6480902
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

smQQLVLDPGFLGLEPLLDLLLGVHQELGASELAQDKYVADFLQWAEPIVVRLKEVRLQRDDFEILKVIGRGAFSEVAVVKMKQTGQ
VYAMKIMNKWDMLKRGEVSCFREERDVLVNGDRRWITQLHFAFQDENYLYLVMEYYVGGDLLTLLSKFGERIPAEMARFYLAEIVMA
IDSVHRLGYVHRDIKPDNILLDRCGHIRLADFGSCLKLRADGTVRSLVAVGTPDYLSPEILQAVGGGPGTGSYGPECDWWALGVFAY
EMFYGQTPFYADSTAETYGKIVHYKEHLSLPLVDEGVPEEARDFIQRLLCPPETRLGRGGAGDFRTHPFFFGLDWDGLRDSVPPFTP
DFEGATDTCNFDLVEDGLTAMVSGGGETLSDIREGAPLGVHLPFVGYSYSCMALRDSEVPGPTP

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Transformation: The construct DNA was transformed into homemade chemically competent cells of the expression strain by a standard heat shock procedure.Glycerol stock prepataion: A number of colonies from the transformation were used to innoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.Expression: A glycerol stock was used to innoculate 10 ml of TB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to innoculate 1L of LB media containing 50 µg/ml kanamycin. After growth at 37°C until the OD600 was around 0.5, the temperature was reduced to 18°C. The cells were induced by the addition of 1.0 mM IPTG when the OD600 was around 0.8. The expression was continued overnight. Two of these 1L growths were combined for the subsequent protein purification.

Purification

Procedure
Purification: The protein was purified by Ni-affinity chromatography, TEV protease digestion to remove the hexahistidine tag, and gel filtration.Column 1: Ni-NTA (2 ml)The clarified supernatent was passed through a column of DE52 resin to bind the DNA and then through a 2 ml volume Ni-NTA column. The Ni-NTA column was washed with Lysis Buffer, and then eluted with Binding Buffer containing 30 mM, then 50 mM, then 250 mM imidazole. The DMPK1A appeared in both the 50 mM and 250 mM imidazole elution fractions.Column 2: S200 16/60 Gel filtration.The GF column was pre-equilibrated with GF Buffer. The TEV protease digested Ni-NTA eluant was concentrated to a volume of about 8 ml. Two gel filtration runs were made, each loading half of the concentrated Ni-NTA eluant. The flow rate was 1.0 ml/min. Eluted proteins were collected in 1.8 ml fractions. The fractions containing protein were identified on a coomasie blue stained gel.

Extraction

Procedure
Cell harvest: Cells were spun down, resuspended in Lysis Buffer, and frozen at -20°C.Cell Lysis: The resuspended cells were thawed and lysed by sonication. The cell debris was spun down.
Concentration: The DMPK1A was exchanged into a buffer of 50 mM BisTrisPropane pH 6.5, 100 mM KCl, 0.5 mM TCEP and concentrated to 11 mg/ml (measured using a nanodrop machine) . The inhibitor BIM 8 (bisindolylmaleimide VIII, acetate salt) was added to a concentration of 0.5 mM.
Ligand
BIM 8 (bisindolylmaleimide VIII, acetate salt)**MassSpec:**Measured: 46061; Expected: 46059
Crystallization: Crystals grew from a 3:1 ratio of protein to precipitant solution (15% PEG3350, 0.2M $MgCl_2$, 0.1M BisTris pH 6.3), using the vapour diffusion method.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected by equilibration into precipitant solution containing 25% ethylene glycol, and then flash frozen in liquid nitrogen. Data was collected at the Swiss Light Source beamline X10. Resolution: 2.8 Å.
Data Processing: