DAPK3: Human Death Associated Kinase 3 (zipper interacting protein kinase - ZIPK)

PDB:2J90

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|4557511
Entry Clone Source: FivePrime
SGC Clone Accession:
Tag:N-terminal his6 tag with TEV cleavage site.
Host:BL21 (DE3) R3 (Phage resistant strain)

Construct

Prelude:
Sequence:

```
mhhhhhhssgvdlgtenlyfq^smVEDHY EMGEELGSGQFAIVRKCRQKGTGKEYAAK FIKKRRLSSSRRGVSREEIEREVNILR
EI RHPNIITLHDIFENKTDVVLILELVSGGE LFDFLAEKESLTEDEATQFLKQILDGVHY LHSKRIAHFDLKPENIMLLDKNVP
NPRIK LIDFGIAHKIEAGNEFKNIFGTPEFVAPE IVNYEPLGLEADMWSIGVITYILLSGASP FLGETKQETLTNISAVNYDFD
EEYFSNTS ELAKDFIRRLLVKDPKRRMTIAQSLEHSW IKAIRRRNVRGEDSG
```

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:1ml from a 20 ml overnight was used to inoculate 1 l of TB medium containing 50µg/ml Kanamycine . E .coli cells were grown in 2.5-L baffled flasks at 37°C until OD reached 2.0. The cells were cooled to 25°C and expression of DAPK3 was induced adding 0.5 mM IPTG at an OD600 of 2.2

Purification

Procedure
Column 1 : : Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)
Procedure: The cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280 was automatically collected.

Column 2 : Gelfiltration, Hiload 16/60 Superdex 200 prep grade, 120 ml (GE/ Amersham Bioscience)
Procedure: The eluted fractions from the Ni-affinity Histrap column were loaded on the gel filtration column at 0.80 ml/min. Eluted proteins were collected in 2 ml fractions.

Concentration : 5 mM DTT was added to the diluted sample before concentrating the protein in Amicon (30 K) to 10.3 mg/ml. The protein concentration was determined spectrophotometrically using the calculated molar extinction coefficient.

Extraction

Procedure
Frozen cell pellets were thawed at 37°C and re-suspended in a total volume of 100 ml lysis buffer. The cells were disrupted by a high pressure cell disrupter (20 kpsi). Nucleic acids and cell debris were removed by adding 0.15% PEI , followed by centrifugation for 30 minutes at 40 000xg. The supernatant was further clarified by filtration (0.2 µm).
Concentration:
Ligand
MassSpec: The mass of the recombinant protein was in agreement with the mass calculated from the expected sequence.
Crystallization: Crystals were grown by vapor diffusion at 4°C from a sitting drop consisting of 150 nl protein (10.3 mg/ml)containing 1 mM Pyridone 6 (2-(1,1-Dimethylethyl)-9-fluoro-3,6-dihydro-7H-benz[h]-imidaz[4,5-f]isoquinolin-7-one) and 50 nl well solution. The drop was equilibrated against well solution containing 30 % PEG1000, 0.1M SPG buffer, pH8.0. The crystal was transferred to a cryo-protectant composed of 20% ethylene glycol before flash-cooling in liquid nitrogen .
NMR Spectroscopy:
Data Collection: Resolution: 2.0 Å diffraction data were collected at the Swiss light source (SLS) beam line BM10.
Data Processing: