Human choline kinase A

PDB:2I7Q

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:34192558
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal histag
Host:E.coli BL21 (DE3) codon plus RIL.

Construct

Prelude:
Sequence:

```
mgsshhhhhhssglvprgsPQPPADEQPEPRTRRRAYLWCKEFLPGAWRGLREDEFHISVIRGGLSNMLFQCSLPDTTATLGDEPRK
VLLRLYGAILQMRSCNKEGSEQAQKENEFQGAEAMVLESVMFAILAERSLGPKLYGIFPQGRLEQFIPSRRLDTEELSLPDISAEIA
EKMATFHGMKMPFNKEPKWLFGTMEKYLKEVLRIKFTEESRIKKLHKLLSYNLPLELENLRSLLESTPSPVVFCHNDCQEGNILLLE
GRENSEKQKLMLIDFEYSSYNYRGFDIGNHFCEWMYDYSYEKYPFFRANIRKYPTKKQQLHFISSYLPAFQNDFENLSTEEKSIIKE
EMLLEVNRFALASHFLWGLWSIVQAKISSIEFGYMDYAQARFDAYFHQKRKLGV
```

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
Column 1: DE52 column
Column 2: 3 mL Ni-NTA column (Qiagen)
Column 3: Superdex 200 column (26x60, Amersham Biosciences)

The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 3 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4°C. The Ni-NTA column was washed with 150 mL of the wash buffer and the protein was eluted with 15 mL of the elution buffer. The protein were further purified and desalted using gel filtration column, Superdex 200 (26/60), which was pre-equilibrated with Gel filtration buffer.

Concentration: All proteins were concentrated using an Amicon Ultra centrifugal filter to a final concentration of 30 mg/mL after the addition of 5mM GDP. Protein concentrations were measured using Bradford assay with purity >95% based on SDS-PAGE analysis.

Extraction

Procedure

Concentration:
Ligand
MassSpec:
Crystallization: Crystallization trials were set up using the sitting drop vapor diffusion method. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 25% PEG3350, 0.1M Ammonium sulfate, 0.1M bis-Tris, pH 6.5, vapor diffusion, sitting drop, temperature 291K.
NMR Spectroscopy:
Data Collection: Resolution: 1.90Å, X-ray source: The Industrial Macromolecular Crystallography Association 17-ID beamline at the Advanced Photon Source.
Data Processing: