ChkB: Human choline kinase beta

PDB:2IG7

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:6978649
Entry Clone Source: GenScript
SGC Clone Accession:
Tag:N-terminal HisTag with integrated thrombin cleavage site (*): mgsshhhhhhssglvpr(*)gs
Host:E.coli BL21 (DE3) codon plus RIL.

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsVGGCLAKDGLQQSKCPDTTPKRRRASSLSRDAERRAYQWCREYLGGAWRRVQPEELRVYPVSGGLSNL
LFRCSLPDHLPSVGEEPREVLLRLYGAILQGVDSLVLESVMFAILAERSLGPQLYGVFPEGRLEQYIPSRPLKTQELREPVLSAAIA
TKMAQFHGMEMPFTKEPHWLFGTMERYLKQIQDLPPTGLPEMNLLEMYSLKDEMGNLRKLLESTPSPVVFCHNDIQEGNILLLSEPE
NADSLMLVDFEYSSYNYRGFDIGNHFCEWVYDYTHEEWPFYKARPTDYPTQEQQLHFIRHYLAEAKKGETLSQEEQRKLEEDLLVEV
SRYALASHFFWGLWSILQASMSTIEFGYLDYAQSRFQFYFQQKGQLTSVHSSS

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: The target was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and chloramphenicol at 37°C. When OD600 was about 3.0, the culture was induced with 1mM IPTG and the temperature was reduced to 15°C, and the cells were allowed to grow overnight before harvesting and flash frozen.

Purification

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

The lysate was centrifuged at 15,000 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 degC. 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
The thawed cell pellets were suspended in 100 mL of the Lysis buffer with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride), and 0.5% CHAPS. The cells were lysed by liquid fluidizer at 20,000 psi.
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 18% PEG3350, 0.2M Ammonium dihydrogen phosphate, 0.1M Hepes pH7.0. Crystals reached a size of about 100 microns within two to three days.
NMR Spectroscopy:
Data Collection: Resolution: 1.58Å
X-ray source: The Industrial Macromolecular Crystallography Association 17-ID beamline at the Advanced Photon Source. Peak data were collected at 0.9797Å.
Data Processing: