CHKB

PDB:3FEG

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 thrombin cleavage site: mgsshhhhhhssglvprgs
Host:BL21 DE3

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsRRRASSLSRDAERRAYQWCREYLGGAWRRVQPEELRVYPVSGGLSNLLFRCSLPDHLPSVGEEPREVL
LRLYGAILQGVDSLVLESVMFAILAERSLGPQLYGVFPEGRLEQYIPSRPLKTQELREPVLSAAIATKMAQFHGMEMPFTKEPHWLF
GTMERYLKQIQDLPPTGLPEMNLLEMYSLKDEMGNLRKLLESTPSPVVFCHNDIQEGNILLLSEPENADSLMLVDFEYSSYNYRGFD
IGNHFCEWVYDYTHEEWPFYKARPTDYPTQEQQLHFIRHYLAEAKKGETLSQEEQRKLEEDLLVEVSRYALASHFFWGLWSILQASM
STIEFGYLDYAQSRFQFYFQQKGQLTSVHSSS

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating freshly transforming E. coli cells (BL21 DE3) into 80 mL of Luria-Bertani medium. After overnight, all of the seeds were inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin at 37°C and grown to an OD600 of 4.0. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20°C in a LEX bubbling system.

Purification

Procedure
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 ºC. 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 proteins were further purified and desalted using gel filtration column, Superdex 200 (26/60), which was pre-equilibrated with Gel filtration buffer.

The protein was concentrated using an Amicon Ultra centrifugal filter at the final concentration of 30 mg/mL. About 20 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were suspended in 100 mL of the binding buffer with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication. The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was used for subsequent steps of purification.
Concentration:30 mg/mL
Ligand
HC-3, ADP**MassSpec:**
Crystallization: The protein was mixed with 3-5 fold molar excess of the inhibitor and ADP, respectively, and incubated overnight at room temperature. Crystals were obtained by the sitting drop vapor diffusion method. 1ul of the protein was mixed with 1 ul of unbuffered reservoir solution consisting of 0.1M sodium cacodylate (pH6.5), 30% polyethylene glycol-4000 and 0.2M ammonium sulfate. Thin plate-like crystals were grown within 3 days. For data collection a single crystal was separated from the cluster and cryoprotected in a 50:50 mixture of Paratone-N and mineral oil before flash cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: