PDXK

PDB:2AJP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_003672
Entry Clone Source:BC000123 (AU11-E6), full length
SGC Clone Accession:PDXK_3:C11
Tag:N-terminal histag with integrated thrombin cleavage site: mgsshhhhhhssglvpr*gs
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsRVLSIQSHVIRGYVGNRAATFPLQVLGFEIDAVNSVQFSNHTGYAHWKGQVLNSDELQELYEGLRLNN
MNKYDYVLTGYTRDKSFLAMVVDIVQELKQQNPRLVYVCDPVLGDKWDGEGSMYVPEDLLPVYKEKVVPLADIITPNQFEAELLSGR
KIHSQEEALRVMDMLHSMGPDTVVITSSDLPSPQGSNYLIVLGSQRRRNPAGSVVMERIRMDIRKVDAVFVGTGDLFAAMLLAWTHK
HPNNLKVACEKTVSTLHHVLQRTIQCAKAQAGEGVRPSPMQLELRMVQSKRDIEDPEIVVQATVL

Vector:p24a-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 37ºC and grown to an OD600 of 3.0. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.5 mM and grown overnight at 20ºC in a LEX bubbling system.

Purification

Procedure
Column 1: DE52 (Whatman) column
Column 2: 3 mL Ni-NTA column (Qiagen)

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 eluate was dialyzed overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol. The protein concentration was estimated based on Bradford assay. Five molar equivalents of ATP[ß,?-NH], 10 mM DTT and 5 mM MgCl2 were added to the purified protein before concentration.

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: The protein was concentrated using an Amicon Ultra centrifugal filter to the final volume of 1 mL and the concentration of 50 mg/mL. About 5 mg of protein was obtained from 1.8 L of cell culture.
Ligand
MassSpec:
Crystallization: Purified His-tagged PDXK was crystallized using the sitting dro vaor diffusion method. Crystals grew in one day when the rotein 15 mg/mL was mixed with the reservoir solution in a 1:1 volume ratio, and the dro was equilibrated against a reservoir solution containing 40% PEG 550MME, 0.1 M glycine, H 9.5.
NMR Spectroscopy:
Data Collection:
Data Processing: