PANK3

PDB:3SMS

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC013705
Entry Clone Source:MGC: AT20-B6
SGC Clone Accession:HPC03H-C09
Tag:N-terminal His6-tag, not removed
Host:BL21-V2R

Construct

Prelude:PANK3: S10-D372
Sequence:

mgsshhhhhhssglvprgsSFPWFGMDIGGTLVKLSYFEPIDITAEEEQEEVESLKSIRKYLTSNVAYGSTGIRDVHLELKDLTLFG
RRGNLHFIRFPTQDLPTFIQMGRDKNFSTLQTVLCATGGGAYKFEKDFRTIGNLHLHKLDELDCLVKGLLYIDSVSFNGQAECYYFA
NASEPERCQKMPFNLDDPYPLLVVNIGSGVSILAVHSKDNYKRVTGTSLGGGTFLGLCSLLTGCESFEEALEMASKGDSTQADKLVR
DIYGGDYERFGLPGWAVASSFGNMIYKEKRESVSKEDLARATLVTITNNIGSVARMCAVNEKINRVVFVGNFLRVNTLSMKLLAYAL
DYWSKGQLKALFLEHEGYFGAVGALLGLPNFSDD

Vector:pET28a-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 at 37°C. When OD600 was ~2.5 to 3.5, the culture was induced with 1mM IPTG and the temperature was reduced to 18°C, and the cells were allowed to grow overnight before harvesting and flash frozen by liquid nitrogen.

Purification

Procedure
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 ºC. The Ni-NTA column was washed with 150 mL of the wash buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The protein were further purified and desalted

using gel filtration column, Superdex 200 (26/60), which was pre-equilibrated with 20 mM Tris pH 8.0, 0.2 M NaCl, and 10 mM DTT. All proteins were concentrated using an Amicon Ultra centrifugal filter to a final concentration of 36 mg/mL. 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 binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole) 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 Microfluidizer (Microfluidics M110-EH).
Concentration:36 mg/mL
Ligand
Adenosine-5'-diphosphate (2R)-N-[3-(heptylamino)-3-oxopropyl]-2,4- dihydroxy-3,3-dimethylbutanamide**MassSpec:**
Crystallization: Crystallization trials were set up using the sitting drop vapor diffusion method and the protein drop was equilibrated against a reservoir solution with 1:1 volume ratio. Prior to crystallization, the purified protein was incubated overnight at 4 °C in the presence of both ligands (5 mM final concentration of each). Crystals of PANK3 were grown at 25% PEG3350, 0.2 M lithium sulfate, 0.1 M Hepes, pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: