PAPSS2

PDB:2AX4

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC009894
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal histag with thrombin cleavage site: mgsshhhhhhssglvprgs
Host:

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsVYQAHHVSRNKRGQVVGTRGGFRGCTVWLTGLSGAGKTTISFALEEYLVSHAIPCYSLDGDNVRHGLN
RNLGFSPGDREENIRRIAEVAKLFADAGLVCITSFISPFAKDRENARKIHESAGLPFFEIFVDAPLNICESRDVKGLYKRARAGEIK
GFTGIDSDYEKPETPERVLKTNLSTVSDCVHQVVELLQEQNIVPYT

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating freshly transforming E. coli cells (BL21 DE3) into 80 mL of Terrific Broth 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 4.0. 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
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 eluate was dialyzed overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol. The protein concentration was measured using Bradford assay. 5mM of ATP and 10 mM MgCl2 were

added to the purified protein before concentration. The protein was concentrated using an Amicon Ultra centrifugal filter to the final concentration of 10 mg/mL. About 15 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 (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 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:
Ligand
MassSpec:
Crystallization: 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 eluate was dialyzed overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol. The protein concentration was measured using Bradford assay. 5mM of ATP and 10 mM MgCl2 were added to the purified protein before concentration. The protein was concentrated using an Amicon Ultra centrifugal filter to the final concentration of 10 mg/mL. About 15 mg of protein was obtained from 1.8 L of cell culture.
NMR Spectroscopy:
Data Collection:
Data Processing: