PWWP2B

PDB:4LD6

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:46250266
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:E.coli BL21 (DE3) pRARE-V2R

Construct

Prelude:
Sequence:

gQSVSECITEDGRTVAVGDIVWGKIHGFPWWPARVLDISLGQKEDGEPSWREAKVSWFGSPTTSFLSISKLSPFSEFFKLRFNRKKK
GMYRKAITEAANAARHVAPEIRELLTQFET

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:PWWP2B was expressed in E.coli BL21 (DE3) pRARE-V2R in Terrific Broth medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 15oC.

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap column (GE Healthcare), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES, pH 7.4, containing 500 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES pH 7.4, 500 mM NaCl, 250 mM imidazole, 5% glycerol). Eluted protein was loaded onto Superdex200 column (26x60) (GE Healthcare), equilibrated with 20 mM PIPES buffer, pH 6.5, and 250 mM NaCl. TEV protease was added to combined fractions containing PWWP2B. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (GE Healthcare), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with

linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 1.5 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For the purification the cell paste was thawed and resuspended in lysis buffer (50 mM HEPES, pH 7.4, 500 M NaCl, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:10 mg/ml. Enzymatic treatment: TEV
Ligand
MassSpec: Expected MW is 13245.0 Da, measured mass is 13245.3966 Da
Crystallization: Purified PWWP2B was crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution (8 mg/mL) with 1 µl of the reservoir solution containing 25% 3350, 0.2 M ammonium acetate, 0.1 M HEPES, pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: