# PPIG

**PDB:**2GW2

## Revision

**Revision Type:**created
**Revised by:**created
**Revision Date:**created
**Entry Clone Accession:**GI:66933018
**Entry Clone Source:**MGC
**SGC Clone Accession:**ppi64.001.179:G10; plate SDC022 G10
**Tag:**
**Host:**BL21 (DE3) Codon Plus RIL

## Construct

**Prelude:**
**Sequence:**

mgsshhhhhhssglvprgsMGIKVQRPRCFFDIAINNQPAGRVVFELFSDVCPKTCENFRCLCTGEKGTGKSTQKPLHYKSCLFHRV
VKDFMVQGGDFSEGNGRGGESIYGGFFEDESFAVKHNAAFLLSMANRGKDTNGSQFFITTKPTPHLDGHHVVFGQVISGQEVVREIE
NQKTDAASKPFAEVRILSCGELIP

**Vector:**p28a-LIC

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**PPIG mutant K125A/E126A was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37oC to an OD600 of 1.0. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

## Purification

**Procedure**
The crude extract was cleared by centrifugation. The clarified lysate was loaded onto 3 ml Ni-NTA column (Qiagen). The column was washed with 5 CV of 50 mM HEPES buffer, pH 7.5, containing 500 mM NaCl, 5% glycerol and 25 mM imidazole, and the protein was eluted with elution buffer (50 mM HEPES, pH 7.5, 500 mM NaCl, 250 mM imidazole, 5 % glycerol). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 50 mM HEPES buffer, pH 7.5, and 500 mM NaCl, 5% glycerol, 1 mM DTT, at flow rate 3.5 ml/min. Combined fractions containing ppi64.001.179 mutant K125A/E126A were concentrated to 15.5 mg/ml. The purification yield was 55 mg of purified protein per 1 L of culture.

## Extraction

**Procedure**
Cells were harvested by centrifugation at 8,000 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 7.5, 0.5 M NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5% glycerol) with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
**Concentration:**15 mg/mL
**Ligand**
**MassSpec:**xpected MW is 21808.6 Da, measured MW is 21677.9 Da
**Crystallization:**Purified ppiG K125A/E126A was and crystallized using the sitting drop vapor diffusion method at 18 °C by mixing 0.2 µl of the protein solution with 0.2 µl of the reservoir solution containing 2M ammonium sulfate, 0.2M Sodium chloride, 0.1M Hepes, pH 7.5.
**NMR Spectroscopy:**
**Data Collection:**
**Data Processing:**