Tag:N-terminal hexahistidine tag
Host: *BL21-CodonPlus (DE3)-RIL*

Sequence:
MHHHHHHSSGRENLYFQGEVYVSASEHPNHFWIQIVGSRSLQLDKLVNEMTQHYENSVP
EDLTVHVGDIVAAPLPTNGSW

YRARVLGTLENGNLDLYFVDFGDNGDCPLKDLRALRSDFLSLPFQAIECSLARIAPSGDQ
WEEEALDEFDRLTHCADWKP

LVAKISSYVQTGISTWPKIYLYDTSNGKKLDIGLELVHKGYAIELPEGSAGSAGSAGSATG
RARARARGRA
Vector:p28a-MHL

Growth

Procedure:A glycerol stock was used to inoculate 20 mL LB media containing 50 µg/mL kanamycin. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB medium which contained 50 µg/mL kanamycin. The culture was grown in LEX at 37°C to OD600 ~1.2 and was induced with the addition of 0.3 mM IPTG. The temperature was reduced to 16°C and the culture was incubated for a further 16 hours before harvesting the cells.

Purification
Procedure
Column 1: Affinity purification: open Ni-NTA Superflow column.
Column 2: Gel filtration: Superdex 200

The cells were collected and then disrupted by sonication in buffer A [25 mM Tris·HCl (pH 8.0), 500 mM NaCl, 5 mM β-mercaptoethanol, 1 mM PMSF]. The supernatants were loaded onto a Ni-NTA resin gravity column and eluted with buffer B [25 mM Tris·HCl (pH 8.0), 500 mM NaCl, 250 mM imidazole, 5 mM β-mercaptoethanol]. The proteins were further purified by gel-filtration chromatography using Superdex 200 (GE) equilibrated with buffer C [25 mM Tris·HCl (pH 8.0), 150 mM NaCl]. Peak fractions were concentrated by ultrafiltration to 30 mg/mL.

Extraction
Procedure
cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. The cells were homogenised and sonicated on ice with 10second pulses and 30 second rest intervals. The output level was 8.5 for 6 mins. The sample was centrifuge at 16,000rpm for 1 hour. The supernatant was purified by affinity, size exclusion and ion exchange.
Concentration:30mg/ml.
Crystallization: The TDRD2–PIWIL1 crystals were obtained in the mixture solution of 0.2 M ammonium formate, 20% (wt/vol) PEG3350. Before data collection, the crystals were soaked in the reservoir solution supplemented with 20% glycerol before being flash-frozen.