

# PIWIL2

PDB:3QIR

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**BC111751

**Entry Clone Source:**MGC CM30-G11 (BC111751)

**SGC Clone Accession:**PIWIL2\_27; plate JMC037B01

**Tag:**N-terminal tag: MGSSHHHHHHSSGRENLYFQG

**Host:**BL21 (DE3) Codon plus RIL (Stratagene)

## Construct

**Prelude:**

**Sequence:**

MGSSHHHHHHSSRENLYFQGRNDCVLDVMHAIYQQNKEHFQDECTKLLVGNIVITRYNNRTRIDVDWNKTPKDSFTMSDGKEITFL  
EYYSKNYGITVKEEYDQPLLIHRPSEQRQDNHGMLLKGEILLPELSFMTGIPEKMKKDFRAMKDLAQGINLSPKQHHSAL

**Vector:**pET28a-MHL

## Growth

**Medium:**LB (Sigma L7658)

**Antibiotics:**50 µg/mL kanamycin (BioShop Canada KAN 201)

**Procedure:**A 250 mL flask containing LB (Sigma L7658) supplemented with 50 µg/mL kanamycin (BioShop Canada KAN 201) was inoculated from a glycerol stock of the bacteria. The flask was shaken overnight (16 hours) at 250 rpm at 37 °C. Using the Lex system, a 2L bottle (VWR 89000-242) containing 1800 mL of TB (Sigma T0918) supplemented with 1.5% glycerol, 50 ug/ mL kanamycin and 600 µl antifoam 204 (Sigma A-8311) was inoculated with 50 mL overnight LB culture, and incubated at 37 °C. The temperature of the media was reduced to 18 °C one hour prior to induction and induced at OD<sub>600</sub> = 2 with 100 µM isopropyl-thio-β-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 18 °C, and cell pellets collected by centrifugation and frozen at -80 °C.

## Purification

**Procedure**

**IMAC:** Unclarified lysate was mixed with 2-3 mL of Ni-NTA superflow Resin (Qiagen) per 40 mL lysate. The mixture was incubated with mixing for at least 45 minutes at 4 degrees Celsius. The mixture was then loaded onto an empty comLum (BioRad) and washed with 100 mL wash buffer.

**Gel filtration chromatography:** An XK 26x65 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1.0 mL/min. The dialyzed sample from the IMAC step (approx. 15 mL) was loaded onto the column at 1.5 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols). Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

## **Extraction**

### **Procedure**

Frozen cell pellet contained in bags (Beckman 369256) obtained from 2L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 25-40 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 10 minutes total sonication time per pellet.

**Concentration:** Purified proteins were concentrated using 15 mL concentrators with a 10,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 20 mg/mL.

### **Ligand**

#### **MassSpec:**

**Crystallization:** Before crystallization, crystals of human PIWIL2 PAZ domain was grown at 291 K using the hanging drop method by mixing equal volumes of 0.2 M NaI, 20% PEG 3350. The crystals were cryoprotected by cryoprotectant consisting of 100% reservoir solution plus 10% glycerol.

#### **NMR Spectroscopy:**

#### **Data Collection:**

#### **Data Processing:**