

# PHF13

PDB:3O70

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**BC038516

**Entry Clone Source:**MGC AT60-D11 (BC038516)

**SGC Clone Accession:**PHF13\_21; plate JMC026:H02

**Tag:**N-terminal tag: MGSSHHHHHHSSRENLYFQG

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

## Construct

**Prelude:**

**Sequence:**

LVTFCMKPFAGRPMIaCNECHTWIHLSCAKIRKSNVPEVFVCQKCRDS

**Vector:**pET28-MHL

## Growth

**Medium:**LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol and 10µM ZnCl<sub>2</sub>

**Antibiotics:**

**Procedure:**A fresh transformation was used to inoculate 20 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol and 10µM ZnCl<sub>2</sub>. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB growth medium containing 100µM ZnCl<sub>2</sub>. The culture was grown in LEX at 37°C to OD600 of 2.3. The temperature was reduced to 14°C and IPTG-based induction (1mM) was carried out according to the manufacturer's protocol. The culture was incubated for a further 18 hours before harvesting the cells. Cells were harvested by centrifugation and pellets were stored at -80°C.

## Purification

**Procedure**

*Column 1:* Affinity purification, open Ni-NTA column

**Procedure:** The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads (buffer is changed to lysis buffer prior to use) by rocking. After 1 hour incubation at 4°C, the bead mixture was transferred to an empty column and washed with 200 mL of lysis buffer. The protein was eluted using ~20mL EB.

*Column 2: Size Exclusion, HiLoad 16/60 Superdex 75 Prep Grade*

**Procedure:** The elution from the NiNTA column was concentrated using 15 mL concentrators with a 3K Da molecular weight cut-off (Amicon Ultra-15, Millipore). The concentrated protein was loaded onto the size exclusion column at a flow rate of 1 mL/min, and 2 ml fractions were collected. The fractions containing protein were identified on an SDS-PAGE gel. Pool the fractions together and concentrated it using the 15 mL concentrators with a 3K Da molecular weight cut-off (Amicon Ultra-15, Millipore).

## **Extraction**

### **Procedure**

Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (100 watts, 10 minutes total time using 10 second pulses followed by 10 second rest) on ice and samples were centrifuged for 60 min at 70 000xg.

**Concentration:** The final concentration was 13.3 mg/ml. The protein yield was approximately 6.5 mg per litre of bacterial culture.

### **Ligand**

### **MassSpec:**

**Crystallization:** Crystals of PHF13 were grown at 291 K using the sitting drop method by mixing equal volumes of 20% PEG8000, 0.1M CHES pH 9.5, The crystals were cryoprotected by cryoprotectant consisting of 100% reservoir solution and 15% glycerol.

### **NMR Spectroscopy:**

### **Data Collection:**

### **Data Processing:**