= Entry clone accession =
BC047504

= Entry clone source =
MGC 11-B7

= SGC clone accession =
YTC047-B02

= Tag =
N-terminal His6-tag

= Construct comments =
sequence 100% identical

= Construct sequence =
mhhhhhhssgrenlyfqgGSAAGPPLSEDDKLQGAASHVPEGFDPTGPAGLGRPTPGLSQGPGKE
TLESALIALDSEKPKKLRFHPKQLYFSARQGELQKVLLMLVDGIDPNFKMEHQNKRSPLH
AAAEAGHVDICHMLVQAGANIDTCSEDQRTPLMEAAENNHLEAVKYLIKAGALVDPKD
AEGSTCLHLAAKKGHYEVVQYLLSNGQMDVNCQDDGGWTPMIWATEYKHVDLVKLLL
SKGSDINIRDNEENICLHWAAFSGCVDIAEILLAAKCDLHAVNIHGDSPLHIAARENRYDC
VVLFLSRDSDVTLKNKEGETPLQCASLNSQVWSALQMSKALQDSA

DNA sequence has been verified by sequencing

= Vector =
pET28-MHL

= Expression host =
BL21(DE3)V2R-pRARE2

= Growth method =
LEX Bubbling

The target protein was over-expressed in E. coli at 37 degrees C by inoculating 30 mL of overnight culture grown in Luria-Bertani medium into 2 L Terrific Broth medium in the presence of 50 ug/mL kanamycin and 34 ug/mL chloramphenicol. When the OD600 of the culture reached ~1.5, the temperature was lowered to 18 degrees and the culture was induced with 0.25 mM final IPTG concentration. The cells were allowed to grow overnight before harvested by centrifugation (7,000 rpm Beckman JLA-8.1000 rotor 15 min) and flash-frozen in liquid nitrogen and stored at -80 degrees.

= Extraction buffers =
Phosphate-buffered saline pH7.4

= Extraction procedure =
2L native cell pellet was resuspended in a total volume of 200 ml extraction buffer with 1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 10 mins at 5" on 7" off duty cycle at 120W output power.

= Purification buffers =
Washing Buffer: Phosphate-buffered saline, 30 mM imidazole
Elution Buffer: Phosphate-buffered saline, 250 mM imidazole
Gel Filtration Buffer: 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM DTT

= Purification procedure =
The lysate was centrifuged at 16,000 rpm (25,800xg RCF(average) for 60 minutes. The supernatant was supplemented with 6 mL Ni-NTA resin (50% flurry) and incubated on a rotary drum for 1 hour at 4 degrees, then loaded onto Bio-rad gravity column. The beads deposited in the open column were then washed with 50 mL lysis buffer followed by 15 mL washing buffer. Bound proteins were eluted using 15 mL elution buffer. The protein was further purified using gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare)preequilibrated with gel filtration buffer. Fractions containing the target protein were pooled and concentrated by centrifugal filters (Amicon mwco 30kDa). The yield of the protein was about 10 mg per liter bacterial culture, and the purity is above 95% judging from SDS-PAGE.

= Protein stock concentration =
Concentration used for crystallization : 20 mg/mL

= Mass spec =
37778.65 g/mol

= Functional multimerization =
Monomer

= Crystallization =

Crystals used for structure determination were grown at 298K in hanging drop setup by mixing 2 uL protein solution with 2 uL well solution containing: 0.1M BIS-TRIS pH6.5, 3.0M Sodium chloride. The crystals were cryoprotected by immersion in paratone-N and flash-frozen in liquid nitrogen.