= SGC clone accession =
PBC025-C01

= Tag =
N-terminal His6-tag,

= Construct comments =

= Construct sequence =
MHHHHHHSSGRENLYFQGMSRRVVRQSKFRHVFGQAAKADQAYEDIRVSKVTWDSSFC
AVNPKFLAIIVEAGGGGAFIVLPLAKTGRVDKNYPLVTGHTAPVLDIDWCPHNDNVIASA
SDDTTIMVWQIPDYTPMRNITEPIITLEGHSKRVGILSWHPTARNVLLSAGGDNVIIIWNV
GTGEVLLSLDDMHPDVIHSVCWNSNGSLLATTCKDKTLRIIDPRKGQVVAEQARPHEGA
RPLRAVFTADGKLLSTGFSRMSERQLALWDPNNFEEPVALQEMDTSNGVLLPFYDPDSSI
VYLCGKGDSSIRYFEITDEPPFVHYLNTFSSKEPQRGMGFMPKRGLDVSKCEIARFYKLH
ERKCEPIIMTVPRKSDLFQDDLYPDTPGPEPALEADEWLSGQDAEPVLISLRDGYVPPKHR
ELRVTKRN

DNA sequence has been verified by sequencing

= Vector =
pFBOH-MHL

= Expression host =
Spodoptera frugiperda

= Growth method =
Shaker

The recombinant donor vector pFBOH-MHL: CORO6 was transformed into DH10Bac E. coli cells (Invitrogen) to generate recombinant viral DNA.
Sf9 cells (Invitrogen) were transfected with Bacmid DNA using jetPRIME® transfection reagent (PolyPlus Transfection), and recombinant baculovirus particles were recovered.
The recombinant virus sequentially amplified from P1 to P3 viral stocks. Sf9 cells grown in I-Max Insect medium (Wisent Biocenter, Cat.# 301-045-LL) to a density of 4mln/mL and with viability not less than 97% were infected with 10 mL of P3 viral stock for each 1 L of cell culture. Cultured cells were collected when cell viability dropped to 75-80%, normally after 4 days of post-infection time

= Extraction buffers =
20 mM Tris [pH 8.0], 500 mM NaCl with 5% glycerol

= Extraction procedure =
Native cell pellet was resuspended in extraction buffer and the cells disrupted by sonication for 10 mins at 5" on 10" off duty cycle at 90W output power.

= Purification buffers =
loading buffer: 20 mM Tris [pH 8.0], 500 mM NaCl with 5% glycerol
Washing Buffer: 20 mM Tris [pH 8.0], 500 mM NaCl with 5% glycerol, 5 mM imidazole
Elution Buffer: 20 mM Tris [pH 8.0], 500 mM NaCl with 5% glycerol, 250 mM imidazole

= Purification procedure =
The crude extract was cleared by centrifugation. The lysate was loaded onto (1ml/L) Talon Metal Affinity Resin (Cat# 635504 Clontech).
The resin was washed with 50 CV of loading buffer (20mM Tris [pH 8.0], 500 mM NaCl with 5% glycerol) and 5 CV loading buffer with 5mM imidazole.
The protein was eluted with elution buffer (loading buffer with 250 imidazole). The protein was further purified by Superdex200 (GE Healthcare) using 20 mM Tris [pH 7.5], 300 mM NaCl.

= Protein stock concentration =
Concentration used for crystallization : 8.1 mg/mL

= Mass spec =
47964

= Functional multimerization =
Monomer

= Crystallization =
Crystallization of CORO6 was performed in 96 well vapor diffusion sitting drop plates by mixing equal volumes of protein and reservoir solution at 20°C.
Diffracting quality crystals were grown in 30% (w/v) PEG 550 MME, 0.05 M Magnesium Chloride and 0.1 M Hepes [pH 7.4].
Crystals were cryoprotected in reservoir solution supplemented with 10% Glycerol and cryo-cooled in liquid nitrogen.