= SGC clone accession =
WDR91:PBC019-H10

= Tag =
N-terminal His6-tag with TEV cleavage site,

= Construct comments =

= Construct sequence =
MHHHHHHSSGRENLYFQG-
PEQPFIVLGQEEYGEHHSSIMHCRVDCSGRRVASLDVDGVIKVWSFNPIMQTKASSISKSP
LLSLEWATKRDRLLLLGSGVGTVRLYDTEAKKNLCEININDNMPRILSLACSPNGASFVCS
AAAPSLTSQVPGRLLLWDTKTMKQQLQFSLDPEPIAINCTAFNHNGNLLVTGAADGVIRL
FDMQQHECAMSWRAHYGEVYSVEFSYDENTVYSIGEDGKFIQWNIHKSGLKVSEYSLPS
DATGPFVLSGYSGYKQVQVPRGRLFAFDSEGNYMLTCSATGGVIYKLGGDEKVLESCLS
LGGHRAPVVTVDWSTAMDCGTCLTASMDGKIKLTTLLAHKA

DNA sequence has been verified by sequencing

= Vector =
pFBOH-MHL

= Expression host =
Spodoptera frugiperda

= Growth method =
Shaker

The recombinant donor vector pFBOH-MHL: WDR91 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 =
20mM Hepes pH 7.4, 500 mM NaCl with 5% glycerol

= Extraction procedure =
Native cell pellet was resuspended in extraction buffer and the cells disrupted by 2 x freeze thaw cycles

= Purification buffers =
loading buffer: 20mM Tris, pH 8.0, 300mM NaCl, 5% glycerol (for lysis buffer added 0.06% NP40, Benzonase, Potease Inhibitor)
Washing Buffer: 20mM Tris, pH 8.0, 300 mM NaCl with 5% glycerol, 15 mM imidazole
Elution Buffer: 20mM Hepes pH 7.4, 500 mM NaCl with 5% glycerol, 300 mM imidazole

= Purification procedure =
The crude extract was cleared by centrifugation. The lysate was loaded onto 5ml Talon Metal Affinity Resin (Cat# 635504 Clontech).
The resin was washed with 5 CV of loading buffer and 10 CV of washing buffer.
The protein was eluted with elution buffer. The protein was further purified by Superdex200 (GE Healthcare) using 20mM Tris pH8.0, 150mM NaCl.
Purification yield was 3.7 mg of the protein per 8L of culture.

= Protein stock concentration =
Concentration used for crystallization : 8.44 mg/mL

= Mass spec =
measured mass:

= Functional multimerization =
Monomer

= Crystallization =
Crystallization of WDR91 WD-repeat domain 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 12% PEG 20k, 0.1 M MES, [pH 6.5].
Crystals were cryoprotected in reservoir solution supplemented with 20% (v/v) Glycerol and then with Paratone, and cryocooled in liquid nitrogen.