= SGC clone accession =
PBC022-E05

= Tag =
N-terminal His6-tag,

= Construct comments =

= Construct sequence =
MHHHHHHSSGRENLYFQGLQCIHIAEGHTKAVLCVDSTDDLLFTGSKDRTCKVWNLVTG
QEIMSLGGHPNNVVSVKYCNYTSLVFTVSTSYIKVWDIRDSAKCIRTLTSSGQVTLGDAC
SASTSRTVAIPSGENQINQIALNPTGTFLYAASGNAVRMWDLKRFQSTGKLTGHLGPVMC
LTVDQISSGQDLIITGSKDHYIKMFDVTEGALGTVSPTHNFEPPHYDGIEALTIQGDNLFSG
SRDNGIKKWDLTQKDLLQQVPNAHKDWVCALGVVPDHPVLLSGCRGGILKVWNMDTF
MPVGEMKGHDSPINAICVNSTHIFTAADDRTVRIWKA

DNA sequence has been verified by sequencing

= Vector =
pFBOH-MHL

= Expression host =
Spodoptera frugiperda

= Growth method =
Shaker

The recombinant donor vector pFBOH-MHL: KIF21A 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 Tris pH8.0, 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: 20mM Tris pH8.0, 500 mM NaCl with 5% glycerol
Washing Buffer: 20mM Tris pH8.0, 500 mM NaCl with 5% glycerol, 5 mM imidazole
Elution Buffer: 20mM Tris pH8.0, 500 mM NaCl with 5% glycerol, 250 mM imidazole

= Purification procedure =
The crude extract was cleared by centrifugation. The lysate was loaded onto 1ml (1ml/L)Talon Metal Affinity Resin (Cat# 635504 Clontech).
The resin was washed with 50 CV of loading buffer (20mM Tris pH8.0, 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 pH8.0, 500 mM NaCl and 5% Glycerol.
The third purification step was the Source Q using 20mM Tris pH7.5(buffer A)with 1M NaCl (buffer B).
Purification yield was 3.5 mg of the protein per 1L of culture.

= Protein stock concentration =
Concentration used for crystallization : 10.0 mg/mL

= Mass spec =
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= Functional multimerization =
Monomer

= Crystallization =
Crystallization of KIF21A 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 25% (w/v) PEG 3350, 0.2 M Ammonium Acetate, 0.1 M Hepes [pH 7.4].
Crystals were cryoprotected with paratone and cryocooled in liquid nitrogen.