

Structure Human ubiquitin like with PHD and ring finger domains 1, SRA domain, in complex with DNA
PDB Code 6VCS
Entry clone accession
Entry clone source
SGC clone accession SDC125B05
Tag C-terminal tag: ahhhhhh
mPSNHYGPIPGIPVGTMWRFVRVQVSESGVHRPHVAGIHGRSNDGAYSLVLAGGYEDDVDHGN
FFTGTGSGGRDLSGNKRTAE
Conserved sequence QSCDQKLTNTNRALALNCFAPINDQEGAEAKDWRSGKPVRVVRNVKGGKNSKYAPAEGNRY
DGIYKVVKYWPEKGKSGFLV
WRYLLRRDDDEPGPWTKEGKDRIKKLGLTMQYPEGYLEALANahhhhhh

Vector pNIC-CH

Expression host BL21 (DE3) Codon plus RIL (Stratagene)

Growth The protein was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin. Cell were grown at 37 °C to an OD600 of 1.5 and induced by meth isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.2 mM, and incubated overnight at 16 °C. Cell pellets collected by centrifugation and frozen at -80 °C.

Extra Lysis buffer: 20 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% glycerol and 2 mM beta-mercaptoethanol
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Extra Frozen cell pellet was thawed and suspended in lysis buffer. The cells were lysed by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 5 sec pulse at half-maximal frequency (5.0), 7 second rest, for 10 minutes total sonication time per pellet. The lysate was centrifuged at 15000rpm for 1h.

Purification Wash buffer: 20 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol and 25 mM imidazole;
n Elution buffer: 20 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol and 300 mM imidazole;
buffer Gel filtration buffer: 20 mM Tris-HCl pH 7.5, 150 mM NaCl and 1 mM DTT
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Cells were lysed in 20 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% glycerol and 2 mM beta-mercaptoethanol buffer and purified by Ni-NTA agarose chromatography. The protein was diluted and applied onto HiTrap SP chromatography column (GE Healthcare) equilibrated with 20 mM Tris-HCl pH 7.5, 25mM NaCl and 1mM DTT. The proteins were eluted with a linear gradient of 0-50% elution buffer (20 mM Tris-HCl pH 7.5, 1M NaCl and 1 mM DTT). The proteins were further purified by gel filtration Superdex 200 10/300 (GE Healthcare). The gel filtration buffer contains 20 mM Tris-HCl pH 7.5, 150 mM NaCl and 1 mM DTT.

Protein
in
stock
concentration

The purified protein was concentrated to 12 mg mL⁻¹.

Crystallization

To get the complex crystal, the protein was incubated with DNA at a molar ratio of 1:1.5 for 1 h on ice before setting up the crystallization trial. The crystals were obtained in 0.1M Sodium malonate pH 7.0, 12% PEG3350. The crystals were cryo-protected in the reservoir solution supplemented with 20% (v/v) glycerol and flash-frozen in liquid nitrogen.