

Struc Human ubiquitin like with PHD and ring finger domains 1, SRA domain, in complex with DNA
ture

PDB 6VCS
Code

Entry
clone
acces
sion

Entry
clone
sourc
e

SGC

clone SDC125B05
acces
sion

Tag C-terminal tag: ahhhhhh
mPSNHYGPIPGIPVGTMRFRVQVSESGVHRPHVAGIHGRSNDGAYSLVLAGGYEDDVHGN
FFTYTSGGGRDLSGNKRTAE

Cons QSCDQKLTNTNRALALNCFAPINDQEGAEAKDWRSGKPVRVVRNVKGGKNSKYAPAEGRNY
truct DGIYKVVKYWPEKGKSGFLV
seque

nce WRYLLRRDDDEPGPWTKEGKDRIKKLGLTMQYPEGYLEALANahhhhhh

Vector pNIC-CH
r

Expr
essio BL21 (DE3) Codon plus RIL (Stratagene)
n

host

Grow The protein was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the
th 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
od 16 °C. Cell pellets collected by centrifugation and frozen at -80 °C.

Extra

ction **Lysis buffer: 20 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% glycerol and 2 mM beta-
buffe mercaptoethanol**
rs

Extra Frozen cell pellet was thawed and suspended in lysis buffer. The cells were lysed by sonication
ction (Virtis408912, Virsonic) on ice: the sonication protocol was 5 sec pulse at half-maximal frequency
proce (5.0), 7 second rest, for 10 minutes total sonication time per pellet. The lysate was centrifuged at
dure 15000rpm for 1h.

Purifi

catio **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;
buffe **Gel filtration buffer:** 20 mM Tris-HCl pH 7.5, 150 mM NaCl and 1 mM DTT
rs

Cells were lysed in 20 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% glycerol and 2 mM beta-
Purifi mercaptoethanol buffer and purified by Ni-NTA agarose chromatography. The protein was
catio diluted and applied onto HiTrap SP chromatography column (GE Healthcare) equilibrated with
n 20 mM Tris-HCl pH 7.5, 25mM NaCl and 1mM DTT. The proteins were eluted with a linear
proce gradient of 0-50% elution buffer (20 mM Tris-HCl pH 7.5, 1M NaCl and 1 mM DTT). The
dure 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.

Prote
in
stock **The purified protein was concentrated to 12 mg mL-1.**
conc
entrat
ion

Cryst To get the complex crystal, the protein was incubated with DNA at a molar ratio of 1:1.5 for 1 h on ice
alliza before setting up the crystallization trial. The crystals were obtained in 0.1M Sodium malonate pH
tion 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.