WHSC1L1

PDB:4YZ8

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC101717
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG
Host:E.coli BL21 (DE3) V2R-pRARE

Construct

Prelude:
Sequence:

gQRESKEALEIEKNSRKPPPYKHIKANKVIGKVQIQVADLSEIPRCNCKPADENPCGLESECLNRMLQYECHPQVCPAGDRCQNQCF
TKRLYPDAEIIKTERRGWGLRTKRSIKKGEFVNEYVGELIDEEECRLRIKRAHENSVTNFYMLTVTKDRIIDAGPKGNYSRFMNHSC
NPNCETQKWTVNGDVRVGLFALCDIPAGMELTFNYNLDCLGNGRTECHCGADNCSGFLG

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:WHSC1L1 was expressed in E.coli BL21 (DE3) V2R-pRARE cells. The transformed cells were grown in TB (terrific broth) medium in the presence of 50 µg/ml of kanamycin and chloramphenicol at 37oC to an OD600 of 3.0. The expression of WHSC1L1 was induced by addition of isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation and the protein was purified by Ni-NTA column (Qiagen). The eluted protein fraction in 20 mM Tris-HCl, 250 mM NaCl, 250 mM imidazole) was loaded into a gel filtration column (Superdex 200, 16/60, GE Healthcare) which was pre-equilibrated with a buffer containing 200 mM PIPES, 250 mM NaCl, pH 6.5. The protein was then processed by in-house produced TEV protease overnight to remove His tag. The protein was further purified to homogeneity with a HiTrap SP FF (GE Healthcare) column, equilibrated with 50 mM PIPES, 10 mM NaCl, 2 mM DTT, pH 6.5 and eluted with linear gradient of NaCl up to 500 mM concentration. The purified protein was stored in 50 mM Tris-HCl, 20 mM NaCl, 1 mM TCEP, pH 8.5.

Enzymatic treatment: TEV cleavage.

Extraction

Procedure
Cells were harvested by centrifugation at 12, 227 Xg for 10 minutes at 4 oC. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For purification, the cell paste was thawed and resuspended in lysis buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 2 mM mercaptoethanol, 5% glycerol, 20 mM imidazole) with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:5.9 mg/ml
Ligand
MassSpec:expected MW = 26455.1 Da, measured MW= 26455.7275 Da.
Crystallization: Purified WHSC1L1 (5.6 mg/ml) was complexed with S-adenosyl-L-methionine (SAM) (Sigma) at 1:10 molar ratio of protein:SAM and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 0.5 µl of WHSC1L1/SAM solution with 0.5 µl reservoir solution (20% PEG 5K MME, 0.1 M Bis-Tris, pH 6.5) and 0.2 µl sarcosine (0.1 M; Hampton research). The crystals were flash frozen in liquid nitrogen using 15% glycerol as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: