SETD7

PDB:4JDS

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:GI:18139549
Entry Clone Source:MGC
SGC Clone Accession:
Tag:C-terminal noncleavable hexa His-tag
Host:E.coli BL21 (DE3) V2R-pRARE

Construct

Prelude:
Sequence:

mQYKDNIRHGVCWIYYPDGGSLVGEVNEDGEMTGEKIAYVYPDERTALYGKFIDGEMIEGKLATLMSTEEGRPHFELMPGNSVYHFD
KSTSSCISTNALLPDPYESERVYVAESLISSAGEGLFSKVAVGPNTVMSFYNGVRITHQEVDSRDWALNGNTLSLDEETVIDVPEPY
NHVSKYCASLGHKANHSFTPNCIYDMFVHPRFGPIKCIRTLRAVEADEELTVAYGYDHSPPGKSGPEAPEWYQVELKAFQATQQKhh
hhhh

Vector:pET28a-LIC-CHis

Growth

Medium:
Antibiotics:
Procedure:SETD7 was expressed in E.coli BL21 (DE3) V2RpRARE in TB medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 and induced by adding isopropyl-1-thio-D-galactopyranoside (IPTG, final concentration 1 mM) and incubated overnight at 15oC.

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap column (GE Healthcare), charged with Ni2+. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl, 50 mM imidazole and 5% glycerol, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 ml/min. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham

Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 17 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For the purification the cell paste was thawed and resuspended in lysis buffer (phosphate-buffered saline, pH 7.4, 0.25 M NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:87.5 mg/ml
Ligand
PF-5426**MassSpec:**Expected MW is 29779.1 Da, measured mass is 29793.3178 Da.
Crystallization: Purified SETD7 (10 mg/mL) was complexed with PF5426 at 1:5 molar ratio of protein:inhibitor and crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 25% P3350, 0.1 M ammonium sulfate, 0.1 M BisTris, pH 6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: