SETD2

PDB:4FMU

Revision

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Entry Clone Accession:GI:197313748
Entry Clone Source:MGC
SGC Clone Accession:SETD2:JMC009-H07:C43809
Tag:N-terminal: His-tag with integrated TEV protease site:MHHHHHHSSGRENLYFQG
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gETSVPPGSALVGPSCVMDDFRDPQRWKECAKQGKMPCYFDLIEENVYLTERKKNKSHRDIKRMQCECTPLSKDERAQGEIACGEDC
LNRLLMIECSSRCPNGDYCSNRRFQRKQHADVEVILTEKKGWGLRAAKDLPSNTFVLEYCGEVLDHKEFKARVKEYARNKNIHYYFM
ALKNDEIIDATQKGNCSRFMNHSCEPNCETQKWTVNGQLRVGFFTTKLVPSGSELTFDYQFQRYGKEAQKCFCGSANCRGYLGGENR
VSIRAAGGKMKKERSRK

Vector:pET28-MHL

Growth

Medium:SETD2 was expressed in E.coli BL21(DE3) codon plus RIL in Terrific Broth (TB) medium in the presence of 50 µg/ml ofkanamycin at 37oC to an OD600 of 1.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15oC.
Antibiotics:
Procedure:

Purification

Procedure
The crude extract was cleared by centrifugation. The clarifiedlysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), chargedwith Ni2+. The column was washed with 10 CV of 20 mM HEPES buffer, pH 7.4,containing 250 mM NaCl and 50 mM imidazole, and the protein was eluted with elutionbuffer (20 mM HEPES, pH 7.4, 250 mM NaCl, 250 mM imidazole). The protein wasloaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with20 mM PIPES buffer, pH 6.5, and 250 mM NaCl, at flow rate 4 ml/min. TEV proteasewas added to the pooled fractions to remove His-tag. The protein was further purifiedto

homogeneity by ion-exchange chromatography on Source 30S column (10x10)(Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted withlinear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 40mg of the protein per 1L of culture.

TEV cleavage.

Extraction

Procedure
Cells were harvested by centrifugation at 12,227 Xg. The cell pellets were frozen in liquid nitrogen and stored at -80°C. For thepurification, 11 g of the cell paste was thawed and resuspended in 110 ml lysis buffer(50 mM HEPES, pH 7.4, 500 mM NaCl, 5 mM imidazol, 2 mM ß-mercaptoethanol, 5%glycerol) with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). Thecells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:26.1 mg/ml
Ligand
Sinefungin analog**MassSpec:**expected MW = 31902.3 Da, measured MW= 31901.6 Da.
Crystallization: Purified SETD2 (10.3 mg/ml) was complexed with Pr-SNF at 1:5molar ratio of protein:compound and crystallized using the sitting drop vapor diffusionmethod at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solutioncontaining 20 % PEG4000, 10 % isoprpanel, 0.1 M HEPES, pH 7.5.
NMR Spectroscopy:
Data Collection:
Data Processing: