SETD2

PDB:4H12

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_054878
Entry Clone Source:MGC
SGC Clone Accession:GI:197313748
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:
Antibiotics:
Procedure:SETD2 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) medium in the presence of 50 µg/mL of kanamycin at 37 ºC 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 15 ºC.

Purification

Procedure
The crude extract was cleared by centrifugation. The clarified lysate was loaded onto 5 mL HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of wash buffer, and the protein was eluted with elution buffer. 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 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 40 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 12, 227 Xg. The cell pellets were frozen in liquid nitrogen and stored at -80 ºC. For the purification, 11 g of the cell paste was thawed and resuspended in 110 mL lysis buffer with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:26.1 mg/ml
Ligand
MassSpec: Expected MW = 31902.3 Da.
Measured MW= 31901.6 Da.
Crystallization: Purified SETD2 (10.3 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 1 µl of the protein solution with 1 µl of the reservoir solution containing 30% PEG 2K MME, 0.1 M KSCN.
NMR Spectroscopy:
Data Collection:
Data Processing: