

SETD3

PDB:3SMT

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:

Entry Clone Source:MGC

SGC Clone Accession:JMC01HG05

Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG

Host:E.coli BL21 (DE3) V2R-pRARE

Construct

Prelude:

Sequence:

gGKSRVKTQKSGTGATATSPKEILNLSELLQKCSSPAPGPGKEWEYYVQIRTLVEKIRKKQKGLSVTFDGKREDYFPDLMKWAS
ENGASVEGFEMVNFKEEGFGLRATRDIKAEEFLWVPRKLLMTVESAKNSVLGPLYSQDRILQAMGNIALAFHLLCERASPNSFWQP
YIQTLPSEYDTPLYFEEDDEVRYLQSTQAIHDVFSQYKNTARQYAYFYKVIQTHPHANKLPLKDSFTYEDYRWA
VSSVMTRQNQIPTE
DGSRVTALIPLWDMCNHTNGLITTGYNLEDDRCEVALQDFRAGEQIYIFYGTRSNAEFVIHSGFFF
DNNSHDRVKIKLGVSKSDR
LYAMKAEVLRAGIPTSSVFAHFTEPPISAQLLAFLRVFCMTEELKEHLLGDSAIDRIFTLGNSEFPV
SDNEVKLWTFLEDRAS
LLLKYKTTIEEDKSVLKNHDL
SVRAKMAIKLRLGEKEILEKAVKSAAVNREYYRQQMEEKAP

Vector:pET28-MHL

Growth

Medium:BL21 (DE3) V2R_pRARE in M9 minimal medium

Antibiotics:50 µg/ml of kanamycin

Procedure:SETD3 was expressed in E.coli BL21 (DE3) V2R_pRARE in M9 minimal medium in the presence of 50 µg/ml of kanamycin. Cell were grown at 37°C to an OD₆₀₀ of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15°C.

Purification

Procedure

The lysate was loaded onto 5 ml HiTrap column (Amersham Biosciences), 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. TEV protease was added to combined

fractions containing SETD3 and incubated overnight at 4oC. 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 (01mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi

Concentration: 23 mg/ml

Ligand

MassSpec: Enzymatic treatment: TEV - Expected MW is 57457.9 Da, measured mass is 57459.9233 Da.

Crystallization: Purified SETD3 (10.8 mg/mL) was complexed with S-adenosyl-L-methionine (SAM) (Sigma) at 1:10 molar ratio of protein:SAM and crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 μ l of the protein solution with 1 μ l of the reservoir solution containing 20 % PEG4000, 0.2 M calcium Acetate, 0.1 M sodium cacodalyte, pH 6.5

NMR Spectroscopy:

Data Collection:

Data Processing: