

# 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:**

gGKKSrvKTQKSGTGATATVSPKEILNLTSELLQKCSSPAPGPGKEWEEYVQIRTLVEKIRKKQKGLSVTFDGKREDYFPDLMKwas  
ENGASVEGFEMVNFKEEGFLRATrdIKAEELFLWVPRKLLMTVESAKNSVLGPLYSQDRILQAMGNIALAFHLLCERASPNSFWQP  
YIQTLPSEYDTPLYFEEDEVRYLQSTQAIHDVFSQYKNTARQYAYFYKVIQTHPHANKLPLKDSFTYEDYRWAVSSVMTRQNQIPTE  
DGSrVTLALiPLWDMCNHTNGLITTGYNLEDDRCECVAlQDFRAGEQIYIFYGTRsNAEFVIHSGFFFDNNSHDrVKIKLGVSKSDR  
LYAMKAeVLARAGIPTSSVFALHfTEPPISaQLLaFLRVfCMTEELKEHLLGDSaIDRIFTLGnSEfPVSWDNEVKLWTFLEDRAS  
LLlKTYKTTIEEDKSVLKNHDLsVRakMaIKLRLGKeiLEKAVKsAAVNREYYRQQMEEKAP

**Vector:**pET28-MHL

## Growth

**Medium:**BL21 (DE3) V2RpRARE in M9 minimal medium

**Antibiotics:**50 µg/ml of kanamycin

**Procedure:**SETD3 was expressed in E.coli BL21 (DE3) V2RpRARE 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 Ni<sup>2+</sup>. 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 4°C. 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 imidazole, 2 mM  $\beta$ -mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (0.1 mM 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  $\mu$ l of the protein solution with 1  $\mu$ l of the reservoir solution containing 20 % PEG4000, 0.2 M calcium Acetate, 0.1 M sodium cacodylate, pH 6.5

### **NMR Spectroscopy:**

### **Data Collection:**

### **Data Processing:**