SETDB1

PDB:3DLM

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_036564
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated TEV protease site
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene).

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQGDLIVSMRILGKKRTKTWHKGTLIAIQTVGPGKKYKVKFDNKGKSLLSGNHIAYDYHPPADKLYVGSRVV
AKYKDGNQVWLYAGIVAETPNVKNKLRFLIFFDDGYASYVTQSELYPICRPLKKTWEDIEDISCRDFIEEYVTAYPNRPMVLLKSGQ
LIKTEWEGTWWKSRVEEVDGSLVRILFLDDKRCEWIYRGSTRLEPMFSMKT

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:SETDB1 Tudor domain was expressed in E.coli BL21 (DE3) codon plus in M9 minimal medium in the presence of 50 μg/ml of kanamycin. Cell were grown at 37 degC 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 degC.

Purification

Procedure
The lysate was loaded onto 5 ml HiTrap 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 HEPES buffer, pH 7.4, and 250 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 HEPES, pH 7.4, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 1 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 with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:9 mg/ml
Ligand
MassSpec: Expected MW for SeMet labelled protein is 26528.28 Da, measured mass is 26680.87 Da.
Crystallization: Purified SETDB1 was crystallized using hanging drop vapor diffusion method at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 20 % PEG 3350, 0.2 M di-Na Tartrate.
NMR Spectroscopy:
Data Collection:
Data Processing: